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INVESTIGATION OF METHODS FOR INTRODUCING ANTIOXIDANTS INTO FOODS

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ANTIOXIDANTS	FOOD PREPARATION	STUDIES	FREEZE-DRIED FOODS
ANTIOXIDATION	FREEZING	POLARITY	OXIDATION
FOOD	LIPIDS	VOLATILITY	ACCEPTABILITY
FOOD PROCESSING	AQUEOUS SOLUTION	STORAGE	SOLIDS

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Methods for introducing antioxidants into food systems were assessed by the use of radioactive antioxidants. Propyl gallate, BHT, BHA, EDTA or citric acid were each applied to breaded chicken legs, breaded fish sticks and frankfurters by a variety of methods, and retention of the antioxidant through subsequent processing including freezing was determined. Penetration of the antioxidant was measured, and association of the antioxidant with free lipid, bound lipid, aqueous and solid fractions was determined. Each antioxidant was applied by various methods to pork pieces, beef stew and carrots which were ultimately

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freeze-dried. Retention through freeze-drying and association with various fractions were determined. Behavior was influenced by method of application and subsequent processing. Most of the observations could be rationalized on the basis of polarity and volatility considerations.

Six month storage was conducted on each of the products. Phenolic and chelating type antioxidants were selected for each product and applied by one method. Sensory evaluations and chemical tests for oxidative deterioration were performed monthly. Only with frankfurters were the antioxidants effective in delaying onset of lipid oxidation and extending storage life. Chicken legs and fish sticks both with and without antioxidant were acceptable after 6 months at -20°C. All freeze-dried products were unacceptable after one month in air at 32°C and antioxidants were not effective in extending storage life.

PREFACE

The shelf life of many convenience foods, such as precooked frozen and precooked freeze-dried items, is limited by auto-oxidation of the lipids with consequent development of rancidity. Use of antioxidants to retard this degradation has not been rewarding, in part from a lack of knowledge of the fate of the antioxidant after application to the food. A study was designed to determine the location and retention of several phenolic antioxidants and metal inactivators in a variety of frozen and freeze-dried foods as functions of mode of application and subsequent processing. These determinations were facilitated by the use of radioactive labels on the additives. Using the antioxidant systems, including mode of application, providing optimal location and retention in the food, efficacy of the antioxidant system was determined by a storage study.

The work reported here was performed in the Department of Food Sciences, University of Wisconsin - Madison, under Contract Number DAAG17-73-C-0214 for the period May 1973 to March 1975. The investigators were Robert C. Lindsay and Daryl B. Lund. The collaborators were Alfred L. Branen, H. C. Chang, Sally E. Dunnick and James A. Steinke.

The US Army Natick Development Center's Project Officer was A. S. Henick, and the Alternate Project Officer was W. L. Porter, both of the Food Sciences Laboratory.

The assistance of the following is acknowledged and appreciated: The University of Wisconsin - Madison Muscle Biology Laboratory for preparation of frankfurters; the Dow Chemical Company, Midland, Michigan, for supplying food-grade calcium-disodium EDTA: the DuPont Freon Products Division, Wilmington, Delaware, for supplying Freon[R] Food Freezant; Eastman Chemical, Inc., Kingsport, Tennessee, for food-grade BHA, BHT, and propyl gallate.

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I. Introduction

A. General

Antioxidants are used extensively in processed foods to preserve freshness through inhibition of oxidation of lipid components. While the efficacy of antioxidant systems is well established in foods, most systems have been developed and applied through implementation of expected functional antioxidant activity and synergistic antioxidant behavior found through trial and error discovery. The literature is replete with studies on antioxidants and methods of delivery, and several excellent reviews are available (c.f. 1, 2, 3, 4). The reader is referred to these references for a more complete discussion on mechanism of lipid oxidation, role of antioxidants and utilization of antioxidants in food systems.

Although much work has been done on lipid oxidation and the role of antioxidants, little definitive information has been applied to extending the storage life of food products containing lipids which are highly susceptible to oxidation. Such factors as the specific location of the antioxidant to provide lipid oxidation protection or polarity considerations of the antioxidant have not been adequately explored, and it seems obvious that methods of application of antioxidants must take into account these factors.

To provide some information on these critical factors this study was undertaken. The study was designed to evaluate various methods of application of two types of antioxidants, and the evaluation included assessing the effect of the method of application on (1) retention of antioxidant through subsequent processing, (2) penetration of antioxidant within the food system, and (3) distribution of antioxidant into various fractions (free lipid, bound lipid, aqueous and solid). To facilitate measurement of the antioxidant, radioactively labelled antioxidants were used. The two types of antioxidsnts used in this study were (1) the phenolic type (a) BHT (butylated hydroxytoluene; 2,6-di-*tert*-butyl-4-methylphenol M.P. 69-70 C), BHA (butylated hydroxyanisole; 2 or 3-*tert*-butyl-4-methoxyphenol, M.P. 59-60 C), (c) PG (propyl gallate; propyl 3,4,5-trihydroxy benzoate, M.P. 150 C), and (2) the water-soluble, chelating type (a) EDTA (ethylenediaminetetraacetic acid, disodium-monocalcium salt and (b) CA (citric acid). (Note: hereafter in this report the antioxidants will be referred to by their acronyms - BHT, BHA, PG, EDTA and CA). Various methods of application were studied including those that are in common use within the industry today and some new, innovative methods of delivery for which there is currently no technological data.

Two general classes of food products were chosen for study and within each class three representative foods or food systems were investigated. The choice of the specific food systems for study was

based on their susceptibility to lipid oxidation and the fact that their shelf-life was usually determined by lipid oxidation. The two classes of foods were high A_w foods which required storage at freezer temperature and low A_w foods which were freeze-dried. The high A_w foods chosen for study were: (1) breaded, precooked chicken legs, (2) breaded, prefried, fish sticks, and (3) frankfurters. The low A_w foods were: (1) freeze-dried pork pieces, (2) freeze-dried beef stew, and (3) freeze-dried carrots.

In addition to the studies with the labelled antioxidants and the methods of application, a six-month storage study was also completed on each product. From the results of the first part of the investigation, one phenolic and one water soluble, chelating-type antioxidant were chosen for application to the food system. The food system was then subjected to six months' storage and at monthly intervals product was evaluated by a technological panel for flavor and overal acceptability. In addition, selected chemical tests (TBA value, peroxide number and UV absorbance at 232 and 268 nm) were completed on the stored samples at monthly intervals.

B. Objective.

The purpose of this contract was to investigate improved methods for introducing antioxidants into processed foods for the purpose of retarding lipid oxidation during subsequent storage of the foods.

C. Specific Requirements.

The specific requirements for this contract were divided into those that pertain to the initial (Phase I) part and those that pertain to the study (Phase II).

Phase I..

a. The study and investigation required for Phase I shall be comprised of (1) study of methods for the introduction of the selected antioxidants into the selected foods, and (2) determination of the efficacy of the methods studied by measurement of the amount of antioxidant which has become located in each of the several lipid and nonlipid components of each of the selected foods. This work shall be conducted subject to the definitions and limitations set forth below.

(1) The foods to be used for this investigation shall be from those generally recognized as susceptible to lipid oxidation upon storage with consequent development of off-flavors which reduce their acceptability. Not fewer than three foods shall be chosen from each of two classes viz.

(a) high water activity (a_w > 0.90 frozen-stored foods, and (b) low water activity (a_w < 0.35) dry-stored foods. The foods chosen

may be raw or cooked, and may be comprised of any number of ingredients, so long as they are reasonably representative of foods commonly consumed by U. S. military personnel.

(2) Antioxidants shall be chosen from those approved for food use by current regulations of USFDA or USDA, and shall be used in amounts not exceeding those permitted by regulation. Any combination of approved antioxidants, synergists, metal inactivating agents, solvents, diluents, etc. may be used, provided that all substances are approved for food use and that the final concentration of each of the food does not exceed that spproved for food use.

(3) Methods for introducing antioxidant materials into foods may employ any physical state, and any mechanical, biological, physical, chemical or combination process, postmortem, on the food or food ingredient. Methods to be investigated may include, but shall not be limited to, application of vapors, dipping, spraying or pumping of liquids, employment of concentration, electrical or thermal gradients, or any combination of these.

(4) The efficacy of each method, or combination of methods, investigated shall be demonstrated by determination of the kind and quantity of each of the antioxidant substances actually present in the oxidation susceptible lipid sites in the food. These sites shall be considered to be, as separate classes where applicable, (a) the membrane lipids, (b) the micellar lipids, (c) adipose tissue including nonpolar lipids, and (d) the diffuse lipids. The distribution of the total amount of antioxidant applied shall be determined by analysis of each major constituent tissue of the food where practicable and/or difference.

Phase II.

a. The work required for Phase II shall be comprised of (1) selection of the most efficacious method for introducing antioxidant into each of the foods studies in Phase I, (2) improvement of techniques to increase efficacy, and (3) demonstration of improved efficacy by determination of the protective effects of the applied antioxidant during storage of the foods, using as control an unprotected food of the same composition. Definitions and limitations given for Phase I shall be applicable to work conducted in Phase II.

b. Storage of foods for study in Phase II shall be conducted under conditions suitable for each food, viz. (1) for frozen stored foods, well packaged, at temperatures which do not exceed -12 C, and (2) for dry-stored foods, well packaged, at temperatures above 32 C but below 44 C. Neither inert gases nor vacuum shall be used in packaging the foods for storage studies. Storage duration shall be for the lesser of six months or until the food has undergone significant oxidation of its contained lipid. Determination of extent of

oxidation of lipids may be by any suitable chemical, physical or sensory procedures.

II. Materials and Methods

A. Chemicals

Radioactive BHT and BHA were obtained from New England Nuclear Corporation (Boston, Mass.). BHT was labeled with ^{14}C in the methyl groups of the tert-butyl group at a specific activity of 0.875 m Ci/mmole. BHA was randomly labeled with ^{3}H at a specific activity of 320 m Ci/mmole and radiochemical purity was greater than 95%. EDTA tetrasodium salt (prepared with acetic acid-2-^{14}C) with a specific activity of 19.8 m Ci/mmole and citric acid-1,5-^{14}C monohydrate with a specific activity of 18.2 m Ci/mmole were purchased from the Amersham/Searle Corp. (Arlington Heights, Ill.). A total amount of 562 m Ci of propyl gallate (PG) prepared by tritiation with ^{3}H-water was obtained from New England Nuclear Corporation. Radioactive propyl gallate was twice repurified with thin-layer chromatography using Silica Gel G (Brinkmann Instruments, Westbury, N.Y.). Benzene: ethanol (3.5:1, v/v) and benzene were used as developing solvents. The repurified ^{3}H-PG had a specific activity of 664 m Ci/mmole and the radiochemical purity was greater than 90%.

Nonradioactive BHT, BHA and PG were food grade and supplied by Eastman Chemical, Inc. (Kingsport, Tennessee). Food Grade calcium disodium EDTA (salt) was obtained from Dow Chemical Co. (Midland, Mich.) and citric acid from Miles Laboratories (Elkhart, Indiana). Freon[R] Food Freezant (Freon-12) was supplied by DuPont Freon Products Division (Wilmington, Delaware). Reagent grade 2-thiobarbituric acid, 2,6-dichloroquinonechloroimide, 2,2-bipyridine and 2,2-diphenyl-1-picrylhydrazyl (free radical) were obtained from J. T. Baker Co. (Phillipsburg, New Jersey). All routine chemical reagents and solvents were obtained from chemical supply companies.

B. Food Product Preparation

The experiments were conducted in two parts with the initial phase designed to study the penetration and distribution of six radioactive antioxidants applied by various methods to six food systems. These are summarized in Table 1. The second part of the investigation entailed evaluation of the storage stability of each type of product prepared with an antioxidant system applied by a selected method. The selections were based on penetration, distribution and retention data obtained in the first phase, of the study. The products, antioxidants, and methods of antioxidant incorporation for this phase are presented in Table 2.

1. Chicken Legs. For the antioxidant distribution studies frozen

fryer chicken legs (ca. 100 g each) purchased from a local retail market were thawed (at 5°C) and precooked to an internal temperature of 79°C (13-15 min in 100 C steam in an Arnold Sterilizer). For the storage stability studies fresh fryer chicken legs (ca. 100 g each) purchased from a local retail market were precooked to an internal temperature of 79°C (13-15 min in 100 C steam in a still retort).

Individual chicken legs were then battered, breaded (Golden Dipt Co., Millstadt, Ill.),.and fried for 30 sec at 204°C in cottonseed oil (Mr. Chef brand, RE-MI Foods, Inc., Schiller Park, Ill.). After draining excess oil, the legs were frozen in a blast freezer (-26°C). Each chicken leg adsorbed an average of 6.4 gm of batter and 3.9 gm of breading, and reached an average final weight of 98 gm. Fat analyses (Goldfisch) showed the bulk breading contained an average of 0.32% fat, batter 0.83% fat, raw chicken legs 2.6% fat, and fried chicken legs 5.1% fat. Methodology for the incorporation of labelled antioxidants in the distribution studies is described in the radioactive antioxidant application section.

For the storage stability studies, EDTA and BHT were applied by incorporation into the batter mix. Based on the average weight of batter absorbed, the fat content, and the retention of antioxidants in each chicken leg through processing, the steamed chicken legs were dipped into the batter mix containing 0.021% (w/w) EDTA and 0.013% (w/w) BHT. After breading and frying for 30 sec at 204 C in cottonseed oil (Mr. Chef brand, RE-MI Foods, Inc., Schiller Park, Ill.), the chicken legs were individually frozen (-26°C) packaged without vacuum in composite Surlyn^R film pouches, and stored in a freezer at -26°C. The final concentration of each antioxidant was calculated to be approximately 150 ppm based on the fat content of the fried chicken legs. Control samples were also prepsred by similar procedures except antioxidants were omitted. A total of 400 chicken legs were prepared, 200 without antioxidant and 200 with antioxidant.

Immediately prior to taste panel evaluation, samples were removed from the freezer, and two chicken legs were simultaneously thawed in a Tappan microwave oven ("high" setting for three min). Then, the two chicken legs were immediately fried in a Wells Deep Fat Fryer (Model I45, 15-pound fat capacity) for 30 sec at 204°C in vegetable oil (Fry-Wel, Milwaukee, Cheese Co., Milwsukee, WI). The fried chicken legs were drained and the meat from each was portioned into thirds. A section of the meat from each chicken leg was placed in a four-ounce paper sampling cup coded with a three-digit random number.

At the eighth week of storage a commercial fresh sample was introduced into the experiment. Prior to each taste panel, fresh chicken legs were purchased in a local supermarket and were prepared by cooking them to an internal temperature of 79°C followed by

TABLE 1 SUMMARY OF METHODS OF APPLICATION OF RADIOACTIVE ANTIOXIDANTS[1] TO FOOD SYSTEMS

Method of Application		FROZEN FOODS: Chicken legs	FROZEN FOODS: Frank-furters	FROZEN FOODS: Fish sticks	FREEZE-DRIED FOODS: Carrots	FREEZE-DRIED FOODS: Beef stew	FREEZE-DRIED FOODS: Pork chops
Direct Addition	During Mixing		BHA BHT				
	With Salt Cure Mixture		CA BHA EDTA PG BHT				
	During Chopping		CA EDTA BHA PG BHT				
	With Ingredients					CA BHA EDTA PG BHT	
Dipping	Before Cooking					CA BHA EDTA PG BHT	CA BHA EDTA PG BHT
	After Cooking						CA BHA EDTA PG BHT
Spraying	Before Battering	CA BHA EDTA PG BHT		CA BHA EDTA PG BHT			
	Before Freezing	CA BHA EDTA PG BHT		CA BHA EDTA PG BHT	CA BHA EDTA PG BHT		
With Batter		CA BHA EDTA PG BHT		CA BHA EDTA PG BHT			
In Frying Oil		BHT BHA		BHT BHA			
Steam Blanching					BHA		
Immersion Freezing		BHA	BHA	BHA	BHA BHT	BHA	BHA
Freeze-Dry Vacuum Release					BHA BHT	BHA	BHA

[1] CA-Citric Acid; EDTA-Ethylenediaminetetraacetic Acid (Tetra Sodium Salt); BHT-Butylated Hydroxy Toluene; BHA-Butylated Hydroxy Anisole; PG-Propyl Gallate.

TABLE 2 ANTIOXIDANT SYSTEMS AND METHODS OF APPLICATION FOR STORAGE STABILITY STUDIES

Products	Antioxidants Fat Soluble	Antioxidants Water Soluble	Method of Application
Frozen:			
Chicken legs	BHT	EDTA	With batter
Fish sticks	BHA	CA	Spraying before battering
Frankfurters	BHT	--	Direct addition during mixing
	--	EDTA	Direct addition during chopping
Freeze Dried:			
Pork chops	--	CA	Dipping after cooking
	BHA	--	Immersion freezing
Beef stew	BHA	CA	Direct addition with ingredients
Carrots	PG	CA	Spraying before freezing

battering, breading and deep-frying for 45 sec at 204°C in vegetable oil (Fry-Wel). The cooked chicken legs were drained, the meat from each was portioned into thirds, and a section of the meat from each chicken leg was placed in a four-ounce paper sampling cup coded as reference (REF).

2. Fish Sticks. Commercially frozen fish blocks of Atlantic Cod (Gadus Morhus) were obtained (Moore's Seafood, Fort Atkinson, Wis), and were sawed into fish sticks having an average size of 1.3 cm x 2.5 cm x 9.5 cm and an average weight of 27 grams. The frozen fish sticks were dipped in batter (1 part batter solids to 1.75 parts water; Golden Dipt Co., Millstadt, Ill.) for 10 sec, drained 20 sec, breaded (Golden Dipt Co.), and fried for 90 sec in cottonseed oil (Mr. Chef Brand, RE-MI Foods, Inc., Schiller Park, Ill.) at 190 C. After draining excess oil, the fish sticks were frozen in a blast freezer (-26°C). Each fish stick adsorbed about 6-7 grams of batter, took up 1-2 gm of breading, and reached an approximate final weight of 33.5 gm after frying. Fat analyses (Foldfisch) showed the breading contained an average of 0.32% fat, the batter 0.83% fat, raw fish 0.3% fat, raw breaded fish sticks 0.10%, and fried fish sticks 7.40% fat. For antioxidant distribution studies, various levels of radioactive antioxidants were incorporated at the appropriate processing step, and the methodology is described in detail under the section dealing with methods of antioxidant application.

For the storage stability studies, a combination of food grade BHA and citric acid was sprayed onto frozen fish sticks before battering. Based on the results of the distribution studies, the fat content of fish sticks, and the retention of antioxidants through the process, fish sticks were sprayed using two glass atomizers (Brinkmann Instruments, New York) in a manner such that each fish stick received a calculated 0.0096 g of 6.4% (w/w) BHA in corn oil and 0.019 g of 4.0% (w/w) citric acid in propylene glycol. The final concentration of each antioxidant was about 150 ppm based on the fat content of the fish stick. Each fish stick without antioxidants was also sprayed with 0.0096 g of corn oil and 0.019 g of propylene glycol. The sprayed fish sticks were dipped in batter (1 part batter: 1.75 parts water; Golden Dipt Co., Millstadt, Ill.) for 10 sec, drained 20 sec, breaded (Golden Dipt Co.), and fried for 90 sec in cottonseed oil (Mr. Chef brand, RE-MI Foods, Inc., Schiller Park, Ill.) at 190 C. After draining excess oil, the fish sticks were individually frozen in a blast freezer at -26°C. Then, the fried, breaded frozen fish sticks were sealed without vacuum in composite Surlyn® film pouches and stored in a freezer (-26°C). A total of 640 fish sticks were prepared, 320 without antioxidant and 320 with an antioxidant.

Immediately prior to taste panel evaluation samples were removed from the freezer and two fish sticks were simultaneously deep-fried for three minutes at 176°C in vegetable oil (Fry-Wel, Milwaukee Cheese Co., Milwaukee, WI) in a Wells Deep-Fat Fryer (Model I45,

15-pound fat capacity). Each fish stick was then drained, cut in half, and portioned into four-ounce paper sampling cups which were numerically coded with a three-digit random number.

At the 12th week of storage a commercial frozen, precooked Atlantic Cod (Islandic brand) sample was introduced into the experiment. Prior to each panel fish sticks were purchased from a frozen food case in a local supermarket. These fish sticks were deep fried for 1-1/2 minutes in 176°C vegetable oil, drained, cut in half and portioned into four-ounce sampling cups marked as reference (REF).

3. Frankfurters. The following formula was used to prepare the meat mixtures (values in percent): pork (50% fat), 42.52; beef (12.5% fat), 31.42; ice, 20.31; salt-cure mixture, 2.51; corn syrup solids, 1.80; and dextrose-spice mixture, 1.41. Commercial ingredients (Oscar Mayer & Co., Madison, WI) were processed by a procedure similar to that described by Hustad et al. (5). Pork and beef trimmings were pre-ground through a perforated plate (0.32 cm diameter holes) and mixed with other ingredients.

For the antioxidant distribution studies the ingredients were mixed for 8-10 min with a Hobart Mixer (Model K5-A, Ohio) while held at approximately -5°C. The mixtures were then chopped in a Hobart silent chopper (Model 84142, Troy, Ohio) for 12 min. Ice was added during chopping, and a final temperature of about 20°C was attained in the mixture.

The emulsions were stuffed into cellulose casings (Oscar Mayer and Co., Madison, WI), and formed into links (5 inch lengths) of about 48 g each. The links were immersed in liquid smoke for 30 sec at about 21°C, and then rinsed with tap water. The links were heat processed for 20 min at 71°C. After heat processing, the frankfurters were chilled with tap water for 15 min. Casings were removed from the frankfurters, and they were sealed without vacuum in composite Surlyn® film pouches. After packaging, the frankfurters (25% fat) were frozen and stored in a blast freezer (-26°C).

For the storage stability studies meat mixtures were blended in a Rietz ribbon mixer for 10 min. Mixtures were then chopped in a Buffalo silent chopper (Model 23, Buffalo, N. Y.) for 8 min and attained a final temperature of about 13 C. Ice was added only at the start of the chopping process. The emulsions were then stuffed into cellulose casings and formed into links (ca 48 g) approximately five inches in length. The links were immersed 30 sec in liquid smoke (ca 21°C), rinsed in tap water, and processed by gradually heating to 82°C over a one-hour period and holding the frankfurters at 82 C for 15 min. After the frankfurters were cooled to 5°C, the casings were removed and the frankfurters were frozen and stored at -26°C.

For the antioxidant distribution studies, radioactive antioxidants

were incorporated into the frankfurters at various stages of preparation and the methodology is described in the section dealing with methods of antioxidant application.

For the storage stability studies, BHT and EDTA were each added at a concentration of approximately 150 ppm (fat basis). BHT was mixed with the dry ingredients and added during the mixing process. EDTA was added during chopping after the addition of ice. A total of 400 frankfurters were prepared, 200 without antioxidants and 200 with antioxidants.

Immediately prior to taste panel evaluation, eight frozen frankfurters of each treatment were placed in one quart of tap water and heated until the water boiled (approximately 10 min). The samples were then drained, each frankfurter cut into fourths, and a one-quarter section of each frankfurter placed in a coded sampling cup.

At the 8th week of storage a commercial sample was introduced into the experiment. Prior to each panel, frankfurters (Oscar Mayer brand, Madison, WI) were purchased at a local supermarket. To prepare for sensory evaluation, eight frankfurters were placed in one quart of tap water and heated until the water was boiling. These frankfurters were also cut into quarters and a one-fourth section of each frankfurter placed in a paper sampling cup coded as reference (REF).

4. Pork Chops. For antioxidant distribution studies pork chops obtained from a local retail market were hand-cut into 0.65 cm cubes. Pork pieces were then pre-cooked with 100°C steam in an Arnold sterilizer to an internal temperature of 77°C (about 5 min), frozen, and freeze-dried with a Virtis freeze dryer (Model No. 10-010, Gardiner, N. Y.) at a pressure of about 100 microns. Radioactive antioxidants were introduced into the pork pieces at various stages of processing described in the section dealing with application of antioxidants.

For the storage stability studies approximately 500 deboned center sections (30-60 g each) of pork chops approximately 1.3 cm thick (center cut) were cooked with 100°C steam for approximately 13 min to attain an internal temperature of 77°C.

Based on the antioxidant distribution studies, citric acid and BHA were the antioxidants selected for evaluation in the pork chops storage stability studies. After cooling to 4.5 C each pork chop was dipped in a 0.065% (w/w) citric acid solution. Approximately 0.46 g of solution was adsorbed per 28 g (1 ounce) of pork chop resulting in the application of approximately 150 ppm citric acid on a fat basis (an average fat content of 6.9% after cooking). BHA was applied in a Freon^R Food Freezant immersion freezing process by incorporating cottonseed oil (Mr. Chef brand, RE-MI Foods Inc., Schiller Park, Ill.) containing 1.68% (w/w) BHA in the liquid Freon^R Food Freezant (1% w/w). Each 28 g (1 ounce) of pork chop adsorbed

approximately 17.3 mg cottonseed oil resulting in a final concentration of about 150 ppm BHA on a fat basis (an average fat content of 6.9% after cooking). A control sample was processed in a similar manner except antioxidants were not incorporated in the dipping water or in the Freon^R Food Freezant. After freezing, the pork chops were freeze-dried (Portland & Co., Portland, Maine) at a pressure of about 500 microns and were subsequently sealed without vacuum in composite Surlyn^R pouches and stored at 32 C.

Immediately prior to each taste panel evaluation, approximately ten freeze dried pork chop pieces were placed in 2000 ml boiling water and simmered for 30 min. Reconstituted pork chops were then cut into approximately 1.3 cm cubes, mixed, and 4-5 cubes were placed into two-ounce sampling cups numerically coded with three-digit random numbers. At the one-month evaluation, a commercial fresh pork chop reference sample was introduced into the experiment. These samples consisted of deboned center sections from approximately 1.3 cm thick locally purchased fresh pork chops. Each deboned chop was browned for two min on each side in a West Bend Electric Skillet at 163°C. Then one cup of water was added to the skillet and the pork chops were simmered for 45 min. Each chop was cut into about 1.3 cm cubes and 4-5 cubes were portioned into two-ounce sampling cups labeled as reference (REF).

5. Beef Stew. Beef stew was prepared according to the following formula (% w/w): 1.3 cm lean beef cubes, 22.1; hydrogenated shortening (Crisco brand), 1.08; diced onions, 5.9; 0.65 cm diced carrots, 5.9; diced celery, 5.9; frozen peas, 5.9; 0.65 cm diced potatoes, 11.8; water, 41.0; salt, 0.39; and black pepper, 0.02 (6). For the antioxidant distribution studies the meat pieces plus shortening were precooked in an oven at 204°C for 30 min and then the remaining ingredients were added. This mixture was then simmered for 30 min. After cooling the mixture was frozen in thin layers and freeze-dried with a Virtis freeze-drier (Model No. 10-010, Gardiner, N. Y.) at a pressure of about 100 microns. Radioactive antioxidants were added during preparation by procedures described in the section dealing with application of antioxidants.

For the storage stability studies, beef stew was prepared by precooking the meat and shortening at 204°C for 30 min, the remainder of the ingredients were added and the mixture was cooked for 30 min at 82°C. Salt and pepper were then added and the stew was cooled, frozen on trays in a thin layer and dried in a freeze-drier (Portland & Co., Portland, Maine) at a pressure of approximately 500 microns. After drying, samples were sealed without vacuum in composite Surlyn^R film pouches and stored at 32°C. For the sample containing antioxidants, food grade BHA and citric acid were added directly to the stew with the salt and pepper. Sufficient quantities were added so that the final concentration of each antioxidant was 150 ppm based on the fat content of the stew (1.3%).

Prior to each taste panel evaluation, 120 g of each beef stew were reconstituted in 600 ml tap water for ten minutes. After adding 30 g of all-purpose flour (Pillsbury All-Purpose) to each batch of moistened beef stew, the mixtures were placed in double boilers and heated with continuous stirring to a temperature of 85°C (12 to 15 min). Thickened beef stew was portioned into two-ounce servings and placed in two-ounce paper sampling cups coded with three-digit random numbers. At the third month evaluation, a commercial, frozen, reference beef stew sample was introduced into the experiment. The commercial sample (Stouffer's brand frozen beef stew) was purchased in a local supermarket immediately prior to each panel. Following directions on each package, three eight-ounce boil-in-the-bag pouches of the frozen beef stew were heated in boiling water for 25 min. Each package was then opened and the contents of the three packages mixed. The combined beef stew was then portioned into two-ounce servings and placed in two-ounce paper sampling cups coded as reference (REF).

6. Carrots. Carrots purchased from a local grocery store were diced (Urshel Laboratories Dicer, Valparaiso, Ind.) into 0.63 cm cubes. The diced carrots were blanched for 2-1/2 min in a steam blanching tunnel. The carrots were then individually quick-frozen, and dried with a Virtis freeze-drier (Model No. 10-010, Gardiner, N. Y.) at a pressure of about 100 microns. For the antioxidant distribution studies, radioactive antioxidants were transferred to the carrots according to methods described in the section on antioxidant applications.

For the storage stability studies, carrots were sprayed using two glass atomizers (Brinkmann Instruments, N. Y.) in a manner such that each 550 g of carrots received a calculated 0.38 g of 0.078% (w/w) PG in corn oil and 0.49 g of 0.04% (w/w) CA in propylene glycol.

Comparable lots of carrots without antioxidants were also sprayed with 0.38 g of corn oil and 0.49 g of propylene glycol. Processed carrots were individually frozen in a blast freezer (-26°C), and dried in a freeze-drier (Portland & Co., Portland, Maine) at a pressure of about 500 microns for 72 hr. Freeze-dried carrots were sealed without vacuum in composite Surlyn^{R} film pouches and stored at 32°C. The final concentration of each antioxidant was calculated to be approximately 150 ppm based on the average reported β-carotene content of carrots (11,000 IU/100 g and 0.6 ug = 1.0 IU USDA Handbook No. 8, (7)). Forty pounds of processed carrots were prepared, 20 pounds without antioxidant and 20 pounds with antioxidant.

Prior to taste panel evaluation, samples of freeze dried carrots were removed from storage. Approximately 120 g of freeze-dried carrots were soaked in 1000 ml tap water for five min, then drained and placed in 2000 ml of boiling water. The carrots were heated in boiling water for 60 sec with continuous stirring. The carrots were then drained and one-ounce portions were placed into numerically coded two-ounce sampling cups.

At the 12th week of storage, a commercial, canned (Roundy's brand, Madison, WI) diced carrot sample was introduced into the experiment. Prior to the sensory evaluation, the carrots were heated and then drained. One-ounce portions of canned carrots were placed into two-ounce paper sampling cups coded as reference (REF).

C. Application of Radioactive Antioxidants.

Individual radioactive antioxidants were introduced into six food systems by eight distinct procedures. The overall experimental design for the antioxidant distribution and penetration studies is presented in Table 1. The procedures employed in these studies provided the basis for subsequent storage stability studies.

1. Direct Addition. This approach was employed in the manufacture of frankfurters and beef stew.

a. During Mixing. Frankfurters (25% final fat) were prepared by adding 40 u Ci ^{14}C-BHT with 0.113 g unlabeled BHT or 100 u Ci ^{3}H-BHA in 0.113 g unlabeled BHA to each 2.27 Kg of beef-pork mixture during mixing. The anticipated antioxidant concentration in each case was calculated to be 200 ppm based on the fat content.

b. With Salt-Cure Mixture. Frankfurters (25% final fat) were prepared by adding either 10 u Ci ^{14}C-CA, 10 u Ci ^{14}C-EDTA, 20 u Ci ^{14}C-BHT, 50 u Ci ^{3}H-PG or 100 u Ci ^{3}H-BHA with 0.113 g of the corresponding unlabeled antioxidant to the salt-cure mixture used with 2.27 kg of beef-pork mixture for an anticipated level of 200 ppm of antioxidant based on the fat content.

c. During Chopping. Frankfurters (25% final fat) were prepared by adding either 10 u Ci ^{14}C-CA, 10 u Ci ^{14}C-EDTA, 20 u Ci ^{14}C-BHT, 50 u Ci ^{3}H-PG or 30 u Ci ^{3}H-BHA in 0.113 g of the corresponding unlabeled antioxidant to 2.27 kg of beef-pork mixture during the chopping process. The anticipated antioxidant concentration in each case was calculated to be 200 ppm based on the fat content.

d. With Ingredients. Individual batches of beef stew were prepared by adding only labeled antioxidants directly to the stew ingredients prior to cooking. A quantity of the appropriate antioxidant solution was added to each 100 g batch of stew to provide either 10 u Ci of ^{14}C-CA (in propylene glycol), 10 u Ci of ^{14}C-EDTA (in propylene glycol), 2 u Ci of ^{14}C-BHT (in corn oil), 8 u Ci of ^{3}H-BHA (in corn oil) or 8 u Ci of ^{3}H-Pg (in corn oil).

2. Dipping. In this process, ingredients were dipped during preparation of beef stew and pork chops.

a. Before Cooking. Beef stew was prepared by dipping 15 g batches of ingredients into solutions of either ^{14}C-CA (0.3 u Ci/ml propylene glycol), ^{14}C-EDTA (0.3 u Ci/ml propylene glycol), ^{14}C-BHT (0.5 u Ci/ml corn oil), ^{3}H-BHA (0.3 u Ci/ml corn oil), or ^{3}H-PG

(50 u Ci/ml corn oil). After dipping, the 15 g batch was added to another 85 g of beef stew ingredients and the 100 g total then carried through the process.

Pork chop pieces (approximately 0.65 cm cubes) were prepared by dipping 20 g batches into solutions of either ^{14}C-CA (0.3 u Ci/ml propylene glycol), ^{14}C-EDTA (0.3 u Ci/ml propylene glycol), ^{14}C-BHT (0.5 u Ci/ml corn oil), ^{3}H-BHA (5×10^{-3} u Ci/ml corn oil) or ^{3}H-PG (50 u Ci/ml corn oil).

b. After Cooking. Radioactive antioxidants were added to cooked pork chop pieces (approximately 0.65 cm cubes) by dipping batches into the appropriate antioxidant solutions. The antioxidant solutions were similar to those employed for dipping ingredients prior to cooking.

3. Spraying. Radioactive antioxidants were applied to chicken legs, fish sticks and carrots by spraying.

a. Before Battering. Chicken legs and fish sticks were sprayed with solutions containing either radioactive BHT, EDTA, CA, BHA or PG before battering. BHT solutions were prepared by dissolving 40 u Ci of the appropriate radioactive compound and 0.48 g of the corresponding unlabeled compound in 25 ml of propylene glycol. For application to fish sticks, EDTA and CA solutions were prepared by dissolving 40 u Ci of the appropriate radioactive compound and 0.65 g of the corresponding unlabeled compound in 25 ml of propylene glycol. BHA and PG solutions were prepared by dissolving 200 u Ci of the appropriate radioactive compound and 0.5 g of the corresponding unlabeled compound in 25 ml of corn oil. Sufficient quantities of these solutions were then sprayed on the food products with a glass atomizer (Brinkman Inst., N. Y.) so that the calculated final concentration of antioxidant was approximately 200 ppm based on the fat content of the food products (for fat content refer to Food Product Preparation section). For fish sticks, about 0.019 ml propylene glycol or 0.024 ml corn oil was applied per fish stick. With chicken legs, about 0.052 ml propylene glycol or 0.066 ml corn oil was delivered to each chicken leg.

b. Before Freezing. Individual radioactive antioxidants were sprayed onto chicken legs and fish sticks immediately after frying. Solutions similar to those applied to these products before battering were used and sufficient quantities of the appropriate solution were sprayed onto the surface so that the calculated final concentration of antioxidant was approximately 200 ppm based on the fat content of the food.

Carrots were sprayed following blanching but before freezing with either ^{14}C-CA (1.6 u Ci/ml propylene glycol), ^{14}C-EDTA (1.6 u Ci/ml propylene glycol), ^{14}C-BHT (1.6 u Ci/ml corn oil), ^{3}H-BHA (50 u ci/ml corn oil) or ^{3}H-PG (50 u Ci/ml corn oil). The amount applied was

calculated to yield 200 ppm of antioxidant based on the reported average β-carotene content of carrots (11,000 IU/100 g and 0.6 ug = 1.0 IU; USDA Handbook No. 8, (7)).

4. With Batter. This method of antioxidant application was used with fish sticks and chicken legs. A quantity of either 5.2 u Ci ^{14}C-BHT, 1.44 u Ci ^{14}C-EDTA, 1.28 u Ci ^{14}C-CA, 100 u Ci ^{3}H-BHA or 50 u Ci ^{3}H-PG was added to 100 g batter. Sufficient amounts of either unlabeled BHT, EDTA, CA, BHA or PG were then added to bring the final concentration of antioxidant in the batter to 0.007% for fish sticks and 0.014% for chicken legs. Considering the average quantity of batter adsorbed by each fish stick or chicken leg, a final concentration of approximately 200 ppm of antioxidant based on the fat content was deposited. However, each fish stick and chicken leg was weighed before and after battering to obtain the weight of batter adsorbed.

5. In Frying Oil. Either labeled BHA or BHT was added to the cottonseed oil (Mr. Chef brand, RE-MI Foods, Inc., Schiller Park, Ill.) which was used for frying the breaded chicken legs or fish sticks. For BHA, 400 u Ci ^{3}H-BHA and 0.1 g unlabeled BHA were dissolved in 500 ml cottonseed oil, and for BHT, 40 u Ci ^{14}C-BHT and 0.07 g unlabeled BHT were dissolved in 350 ml cottonseed oil. In both cases, the final concentration of antioxidant in the cottonseed oil was 200 ppm.

6. Steam Blanching. BHA was introduced onto carrot pieces by using steam as a carrier. A quantity of 200 u Ci of ^{3}H-BHA and 1.0 g of unlabeled BHA were dissolved in 20 ml of ethanol. Five ml of this solution were then placed in a 100 ml open-ended glass bulb, and the ethanol was evaporated by rotating the bulb and applying mild heat (less than 40°C). BHA was then transferred from the glass bulb to carrot pieces during blanching by passing steam (100°C) through the 100 ml glass bulb and subsequently through a 1.3 cm bed of diced carrots.

7. Immersion Freezing. This method of application was employed for the introduction of either BHA or BHT to each of the six products. The products were frozen in liquid freezant (Freon^{R} Food Freezant) containing corn oil (Mazole brand) and the appropriate labeled antioxidant. Corn oil was dispersed at a level of 1% (w/w) in the Freon^{R} Food Freezant to avoid fat loss from the products during freezing (Kenyon, 8). The food products were immersed in a Freon^{R} Food Freezant: oil bath (-30°C) until the product temperature approached -17.8°C. The products were then removed from the bath, placed in an insulated container and allowed to equilibrate to a final temperature of -17.8°C.

The average initial internal temperature of fish sticks was 44°C, and a 4 min immersion followed by a 1.5 min equilibration was required to reach a final temperature of -17.8°C. For chicken legs, the average initial temperature was 40°C and 17.5 min immersion followed by a 2.0

min equilibration was required to reach -17.8°C. Labeled BHA was introduced into fish sticks and chicken legs by freezing each product in Freon[R] Food Freezant containing dispersed ^{3}H-BHA: corn oil mixture. The final concentration was 0.9 u Ci ^{3}H-BHA per ml Freon[R] Food Freezant: oil mixture. For frankfurters the average initial internal temperature was 20°C and a 5.25 min immersion followed by a 90-sec equilibration was required to reach a final temperature of -17.8°C. The activity of the freezant: oil mixture was 0.03 u Ci ^{3}H-BHA ml.

The procedure for immersion freezing diced (0.63 cubes) carrots average initial temperature of 20°C required a 30 sec immersion in the Freon[R] Food Freezant bath followed by a 90 sec equilibrstion to reach a final temperature of -17.8 C. Amounts of 4.5×10^{-3} u Ci of ^{14}C-BHT/ml of Freon[R] Food Freezant or 0.14 u Ci of ^{3}H-BHA/ml of Freon[R] Food Freezant were used in the experiments.

Similarly, pork chop pieces (0.65 cm cubes) had an average initial temperature of 20°C, and 30-sec immersion in Freon[R] Food Freezant: oil mixture followed by a 90-sec equilibration was required to reach a final temperature of -17.8°C. Twenty g lots of pork chop pieces were frozen, and the concentration of ^{3}H-BHA was 0.14 u Ci per ml Freon[R] Food Freezant: oil mixture. For beef stew the initial temperature was 20 C, and immersion in the Freon[R] Food Freezant: oil bath was 30-sec followed by 90-sec equilibration to -17.8°C. A concentration of 0.13 u Ci of ^{3}H-BHA/ml Freon[R] Food Freezant: oil mixture was employed to treat 100 g lots of beef stew.

8. <u>Freeze Dry Vacuum Release</u>. Individual lots of freeze dried diced carrots (15 g), pork chop pieces (20 g), or beef stew (100 g) were placed in a 2200 cc product chamber and the pressure was reduced to 30 mm Hg absolute. The product chamber was attached to a 100 cc open-ended glass bulb (antioxidant reservoir chamber) which was coated with either BHA or BHT in the concentration and manner previously described for application of antioxidants during steam blanching. A balloon containing approximately 2200 cc of air was attached to the glass bulb and upon opening it to the glass bulb allowed the antioxidant to be vaporized in the glass bulb without creating a potential hazard of escaping radioactive material.

For the antioxidant application, a sufficient quantity of air was contained in the balloon so that when the balloon was opened to the glass bulb and the glass bulb was opened to the product chamber a slight positive pressure was maintained in the system. The antioxidants were transferred via the air stream which pssed from the balloon through the antioxidant reservoir and into the product chamber. The amount of antioxidant transferred depended on the temperature of the artioxidant reservoir, and this was evaluated at 17, 50 and 100°C for BHA and 100°C for BHT.

D. Radioactive Antioxidant Determination.

1. Process Retention and Penetration into Products. The total amount of radioactivity detected immediately after application and that detected after any remaining processes was the basis for calculating the percent antioxidant retention through that process.

For freeze-dried carrots, pork chops, and beef stew, only the retention of antioxidants through each process was determined because the small size of individual pieces precluded sectioning for penetration studies. For frozen products, both retention of antioxidant through processing and penetration of antioxidant into the product were determined. Retention calculationswere similar to those used for freeze dried products.

To demonstrate antioxidant penetration, each product was sectioned into three portions using a razor blade. Each frozen, fried, breaded fish stick was divided into portions consisting of batter and breading, and muscle. The muscle was then divided into an outer and a center portion by removing the outer layer and leaving 1.2 cm x 0.25 cm x 9.4 cm center portion. The edible portion of each frozen, fried, breaded chicken leg was also separated into three portions. These consisted of the batter and bread layer, the skin layer, and the muscle. Frankfurters were each cut into three approximately equal portions consisting of the outer, the intermediate and the center portion.

2. Distribution in Food Fractions. Each food product or portion thereof obtained by the sectioning procedures described above was analyzed for levels of labeled antioxidant in the free lipid, bound lipid, aqueous, and solid fractions. Free lipids were extracted according to a modified procedure of Giam and Dugan (9) and bound lipids were extracted according to a modified procedure of Bligh and Dyer (10).

The Giam and Dugan (9) procedure employs freeze-drying samples prior to Goldfisch extraction with petroleum ether. To prevent possible losses of antioxidants during the freeze-drying step, direct petroleum ether extraction of native samples was employed. Preliminary experiments using ^{14}C-BHT added in batter to fish sticks (3.8×10^5 cpm/fish stick) revealed that the radioactivity in the petroleum ether extract from the Giam and Dugan (9) procedure was similar to that recovered with the modified direct extraction procedure (57.8 and 55.3% of the total, respectively). Additional studies employing similarly prepared fish sticks containing ^{3}H-BHA or ^{3}H-PG were carried out using conditions to minimize losses due to volatility during drying. Samples were held for one week at 21 C in desiccators (760 mm Hg) containing excess anhydrous calcium chloride. For ^{3}H-BHA (2.1×10^6 cpm/fish sticks), the petroleum ether extract contained 73% of the recovered radioactivity for the calcium chloride desiccated samples and 78% using the modified direct (wet) extraction procedure. With ^{3}H-PG

(6.9×10^5 cpm/fish stick), a total of 9% of the radioactivity was in the petroleum ether extract obtained from the desiccated samples and 6% was in the extract obtained from the modified direct (wet) procedure.

In the overall modified procedure for extracting free and bound lipid fractions each sample was placed in a 50 ml glass flask and disintegrated with a microhomogenizer (Virtis "23", Gardiner, N. Y.) at approximately 10,000 rpm for 2 min. The sample was then similarly blended with three consecutive 30 ml portions of petroleum ether each of which was transferred to a 100 ml volumetric flask and finally brought to volume with petroleum ether. Each sample residue was then similarly blended with 25 ml of chloroform: methanol (1:2, v/v) for 2 min. Then 10 ml of chloroform were added followed by blending for 30 sec in the microhomogenizer. Finally, 10 ml of distilled water were added and blending was continued for 30 sec. Each homogenate was filtered with a Hirsch funnel through Whatman No. 1 filter paper. The homogenizer flask was rinsed consecutively with portions of 25 ml chloroform: methanol (1:2), 10 ml of chloroform and 10 ml of distilled water, and each rinse solvent was filtered as previously mentioned. The residue was used in determining labeled antioxidant in the solid fraction. The total filtrate was transferred to a 150 ml separatory funnel, and after standing at least 5 min for phase separation, the chloroform layer was drawn into a 50 ml volumetric flask and brought to volume with chloroform. The aqueous layer was also drawn into a 50 ml volumetric flask and brought to volume with methanol.

3. Counting Procedures. For determination of radioactivity levels in the whole food pieces or solid fractions, a weighed quantity was placed in each glass scintillation vial. Ten ml of scintillator solution (100 g naphthalene, 10 g PPO (2,5-diphenyloxazole), 0.25 g POPOP (1-4-bis-2-5 phenyloxazoly-benzene) in 1000 ml dioxane) were added to each vial and each vial was counted in a Packard Automatic Tri-Carb Spectrometer (Model 3320, Downers Grove, Ill.). Counting periods up to 10 min per sample were used to obtain statistically valid counts. All counts were corrected for quenching using channel ratios. Two ml petroleum ether extract, one ml chloroform extract or one ml aqueous extract were added to each scintillation vial containing 10 ml scintillator solution. These vials were counted using the above procedure.

E. Other Characteristics of Radioactive Antioxidants.

1. Volatility from Frying Oil. Radioactive BHT was added to a 1500 ml quantity of cottonseed oil, and was heated in a deep fryer (Fryryte Model N140E, Nesco Division, The Hoover Co., St. Louis, Mo.) for 6 hr at 206 C. Samples (0.1 ml) were withdrawn periodically to determine residual levels of radioactivity.

2. ^{3}H-Exchange Rates Between Labeled Antioxidants and Water Systems. Stock solutions of ^{3}H-BHA or ^{3}H-PG were prepared by placing

a small amount of either antioxidant in a 250 ml flask, and evaporating carrier solvents with a stream of dry nitrogen gas. The dry antioxidants were each redissolved in a 1 ml of absolute ethanol and subsequently 100 ml of water which had been adjusted to either pH 5.0, 6.0 or 7.0 with HCl or NaOH was added to each flask. The six stoppered samples were held at 5 C for nine weeks and were analyzed periodically for the degree of ^{3}H-exchange.

The amount of exchange was determined by extracting 10 ml aliquots of each stock solution with five consecutive 10 ml quantities of ethyl acetate. Counts were obtained for samples of both aqueous and ethyl acetate phases to determine relative ^{3}H-distribution between phases.

F. Chemical Analysis of Storage Stability Samples.

1. Oxidative Stability Tests.

a. 2-Thiobarbituric Acid (TBA) Numbers. A modification of the Tarladgis *et al.* (11) procedure was used for determining the TBA Number of food products. For the frozen samples approximately 4-5 fish sticks (with antioxidants or control sample), 4-5 chicken legs (with antioxidants or control sample), or 5-6 frankfurters (with antioxidants or control sample) were ground in a Waring Blendor for 5-7 min or until the sample was homogeneous. Ten g of ground fish sticks, chicken legs or frankfurters was blended with 50 ml of distilled water in a Waring Blendor for 2 min. The mixture was transferred quantitatively into a Kjeldahl flask by washing with an additional 47.5 ml distilled water. A 2.5 ml quantity of HCl solution (4 N) and a small amount of antifoaming chemical (1-tetradecanol) were introduced onto the lower neck of the flask, and 2-3 boiling chips were added to prevent bumping. For the freeze dried samples approximately 40-50 g of freeze-dried carrots, beef stew or pork chops were pulverized with a mortar and pestle. Five g of ground freeze-dried carrots or pork chops or 10 g of ground freeze-dried beef stew were transferred into a Kjeldahl flask by washing with 132.5 ml of distilled water. A 2.5 ml quantity of HCl solution (4 N) and a small amount of 1-tetradecanol were added onto the lower neck of the flask, and 2-3 boiling chips were added.

The flasks were placed on a Kjeldahl distillation apparatus and 50 ml of distillate were collected from each. The distillates were filtered and 5 ml of each filtrate was pipetted into a 25 ml glass-stopped test tube. Five ml of TBA reagent (0.02M 2-thiobarbituric acid in 90% glacial acetic acid) were added. The test tubes were stoppered, the contents mixed, and the test tubes were immersed in a boiling water bath for exactly 35 min. After cooling the test tubes in tapwater for 10 min, a portion of each was transferred to a cuvette, and the absorbance of the sample was determined against a distilled water-TBA reagent blank at a wavelength of 538 nm (Beckman Model DK-2, recording spectrophotometer, Fullerton, Calif.). To convert absorbance to TBA number (mg malonaldehyde per 1000 g sample). The

multiplication factor (K) was determined as described by Tarladgis et al. (11). For fish sticks, chicken legs, frankfurters and beef stew, the absorbance at 538 nm was multiplied by the factor 5.1 to yield TBA number. For the freeze dried carrots and pork chops, the absorbance was multiplied by 10.2.

b. Peroxide Values. A modification of the procedure described by Mehlenbacher (12) was used for determination of peroxides in food products. About 100 g of preground fish sticks, chicken legs or frankfurters or about 10 g of preground carrots, beef stew or pork chops were mixed with 350 ml of chloroform for 2 min. The chloroform extract was filtered into a 500 ml flask, and triplicate 25 ml portions of the filtrate were withdrawn for peroxide analysis. Quantities of 37 ml of glacial acetic acid and 1 ml of saturated potassium iodide were added to each 25 ml aliquot of chloroform extract. Solutions were allowed to stand with occasional swirling for exactly one min. Then 30 ml of distilled water and 0.5 ml of 2% starch indicator solution were added, and the mixture was titrated with 0.001 N sodium thiosulfate.

For subsequent calculations, the fat content of each product was gravimetrically determined by initially evaporating the chloroform from 10 ml aliquots of appropriate chloroform extracts in preweighed aluminum pans (1.3 cm x 5.0 cm) at room temperature (approximately 21 C) for 24 hr. This was followed by holding at 121 C in an oven for approximately 5 min, cooling to ambient temperature and weighing. Peroxide values (PV) were calculated using:

$$PV\ (meq/kg\ fat) = ml\ thiosulfate\ used \times 0.001\ N \times \frac{1000}{gm\ fat\ in\ sample}.$$

Peroxide values of the frying oils used to prepare the fish sticks and chicken legs for the storage stability study were monitored during the frying operation. Triplicate one ml samples of oil (0.91 ± 0.02 g) were analyzed (Mehlenbacher, 12) using 0.001 N sodium thiosulfate for titration.

c. UV Absorbance of Lipids. A modification of the procedure reported by Danopoulos and Ninni (13) was used. Ten g of preground fish sticks, chicken legs, frankfurters, carrots, pork chops or beef stew were blended with 250 ml of chloroform-methanol 2:1 (v/v) for 2 min. The extract was filtered, and most of the solvent was removed with a vacuum evaporator (Buchi-Veso, Rinco Instrument Co., Inc., Greenville, Ill.). After transferring the concentrated extract to a separatory funnel containing 1 g of sodium chloride dissolved in 100 ml of distilled water, the lipid was extracted using three 50 ml quantities of redistilled ethyl ether. The combined extracts were washed with 20 ml distilled water, dried by addition of about 15 g anhydrous sodium sulfate and filtered. The filtrate volume was reduced to about 10 ml with a vacuum evaporator. One ml of the ethyl ether concentrate was pipetted into a 10 ml volumetric flask and dried with a nitrogen

stream. The residue wss redissolved in petroleum ether by bringing to volume, and UV absorbances at 232 and 268 nm were measured (Beckman Model DK-2 recording spectrophotometer, Fullerton, Calif.). Additional dilutions with petroleum ether were used if necessary. The weight of lipid in one ml of the ethyl ether concentrate was determined by pipetting one ml of the concentrate into a small preweighed aluminum psn (1.3 cm x 5.0 cm) and allowing the ethyl ether to evsporate at room temperature (approximately 21 C) for 24 hr. Each pan wss then placed in sn oven at 121 C for five min, and after cooling, esch pan was weighed. Using the weight of the lipid, the absorbance values were normalized to a lipid concentration of 1% in petroleum ether by:

$$A(1\%\ \text{lipid solution}) = \frac{A\ (EXP)}{\text{weight of sample (mg)}} \times 10 \times \text{dilution factor}$$

2. <u>Levels of Antioxidsnts</u>. Spectrophotometric measurements of unlabeled phenolic sntioxidsnts and EDTA in experimentsl foods were attempted to indicate levels of antioxidants deposited and/or amounts of chemically unsltered antioxidants remaining. Measurement of citric acid in the foods using gas chromatogrsphic methods was not attempted because of the insensitivity of the method.

a. <u>Phenolic Antioxidants</u>. The spectrophotometric procedure for phenolic antioxidsnts in fats snd oils described by Sahssrabudhe (14) wss employed for anslysis of the experimental foods. Approximately 250 g of either fish sticks, chicken legs, or frsnkfurters were disintegrsted in s Waring Blendor until homogeneous (5-7 min). Samples of esch (approximstely 40 g) were then weighed into Soxhlet extraction thimbles (Whatmsn, 33 x 94 mm), and the samples were dried in sn oven for six hr st 102 C. About 100 g of either freeze-dried csrrots, beef stew and pork chops were ground with s mortsr snd pestle, snd ssmples (approximately 40 g) were weighed into Soxhlet extraction thimbles. For BHA and BHT, dried samples were extracted in a Soxhlet apparatus with 150 ml of chloroform for 10 hr. For PG, 100 ml of 95% ethanol was used in the Soxhlet extraction. During the disintegration step, levels of 150 ppm of the appropriste sntioxidant were sdded directly to food samples initially prepared without sntioxidsnts, and these served as reference stsndsrds for the determinations.

Solvents were removed from each Soxhlet extract under reduced pressure. Samples in esch flask were rinsed with 100 ml of hexsne followed by rinsing with four consecutive 25 ml portions of 80% squeous ethanol. All solvent was transferred to a 250 ml separatory funnel for phase separation. The hexane was further extracted with four consecutive 25 ml portions of acetonitrile. Ethanol and acetonitrile extracts from each sample were combined, and then dried under reduced pressure. The residues were dissolved in a minimum amount of absolute ethanol, transferred to a 10 ml volumetric flask, and brought to volume. Aliquots of these solutions were used for subsequent snalyses.

For thin-layer chromatography most of the ethanol was removed from sample aliquots with a stream of nitrogen before spotting. One dimensional ascending chromatography on plates (20 cm x 20 cm) coated with 1 mm thick silica gel G was used with chloroform as the developing solvent.

After development plates were dried, the reference lane was sprayed with a chloroform solution of 2,2 - diphenyl - 1 - picryl-hydazyl (free radical) for location of the antioxidant spots. Corresponding areas in the analysis lanes were scraped off plates and extracted. For BHA 5 ml of 50% ethanol/water was used and for BHT, 5 ml of absolute ethanol was employed. For PG, 5 ml of 50% ethanol followed by 4 ml 2.5% aqueous ammonium acetate was used. After centrifuging at low speed in a clinical centrifuge, ethanol fractions were transferred to volumetric flasks and brought to 10 ml volumes with 50% ethanol for BHA, 20% aqueous ethanol for BHT and 2.5% aqueous ammonium acetate for PG.

For color development with PG, one ml of ferrous sulfate (0.04% $FeSO_4{:}7H_2O$ in distilled water) was added to 10 ml of extract, and the mixture was allowed to stand for 10 minutes before reading absorbance at 515 nm. For color development with BHA, each 10 ml of 50% aqueous ethanol extract was combined with two ml of 2% sodium borate (Borax) solution and two ml of 0.01% 2,6-dichloroquinonechlorimide in absolute ethanol. After color development for 15 min, five ml of n-butanol was added and absorbance at 620 nm was determined with a Spectronic 20 (Bausch and Lomb). For color development in the BHT analysis, two ml of 2,2-bipyridine solution (200 mg 2,2-bipyridine in 1 ml absolute ethanol, then diluted to 100 ml with water) and two ml of 0.2% Fe $Cl_3.6H_2O$ solution were added to 10 ml aliquots of the ethanol extracts. After standing 30 min in a dark place for color development, five ml of n-butanol was added to each and absorbance was read at 522 nm. Standard curves were prepared for each antioxidant.

b. EDTA. The Dow Laboratories procedure (15) was used for the analysis of EDTA in frankfurters and chicken legs. The edible portion of one chicken leg (ca. 60 g) or one frankfurter (ca. 48 g) was used in each analyses. Each sample was blended in a Waring Blendor (high speed) with 300 ml of distilled water, and then filtered through Whatman No. 1 filter paper. The filtrate was analyzed by pipetting varying amounts (0, 5, 10, 15, 20, and 25 ml) of filtrate into 50 ml volumetric flasks each containing 5 ml of zirconium analytical solution (stock, 2.250 g of zirconium oxychloride ($Zr\ O\ CL_2.8H_2O$) in 65 ml conc. HCl, then diluted to 1000 ml; for use, 10 ml of stock and 5 ml conc. HCl brought to 250 ml), and 5 ml xylenol orange reagent [0.8 g of xylenol orange in 334 ml conc. HCl plus 400 ml hydroxyl-amine hydrochloride solution (100 g/400 ml H_2O)], After allowing one hr for color development, the absorbance of each at 535 nm was determined with a Spectronic 20. A standard curve was prepared with calcium disodium EDTA.

G. Sensory Evaluation of Storage Stability Samples.

Technological sensory evaluation panels employing 15-20 experienced panelists were utilized to monitor sensory characteristics of the samples under standardized conditions (Amerine *et al.*, 16). Panelists were seated in isolated tasting booths equipped with running water available on a free choice basis. Indoor fluorescent lighting was used in the taste panel room.

Each panelist received a tray of samples, a ballot, and utensil at the initiation of each session, and at some sessions a second tray of samples was evaluated. The fish sticks and carrots, or the frankfurters and pork chops, were served during the same tasting session. In each case half the judges received one tray first and half of the judges received the other tray first. The chicken legs and beef stew were evaluated in separate sessions.

All samples were kept warm (ca. 60 C) in a Wells warming table until served, and were evaluated within two hours after preparation.

Detailed preparation procedures for each product are described in the Methods Section entitled "Food Product Preparation." The magnitude estimation ballot (17) required the judges to score each sample for overall acceptance on a horizontal unmarked line ranging from "extremely acceptable" to "extremely unacceptable" (Fig. 1). The judges were also asked to score the intensity of oxidized flavor and intensity of any other off-flavors. Both of these aspects were scored on horizontal scales ranging from "pronounced" to "none". If judges perceived any off-flavors, they were asked to describe the off-flavor at the bottom of the ballot in the space available for comments.

The ballots included both acceptability and intensity scales, and as the experiment progressed it became evident that panelists lacked reference points in positioning their scores for the experimental samples. This was especially true for the freeze dried products, particularly carrots and beef stew. Although the initial experimental design did not incorporate comparison samples of commercially available products, these were ultimately introduced into the experiment for all of the products. Commercial comparison samples were introduced beginning with the fourth week evaluation for freeze-dried pork chops, the eighth week evaluation for frozen chicken and frankfurters and the twelfth week evaluation of frozen fish sticks, freeze-dried carrots and freeze-dried beef stew. While the flavor quality of commercially obtained samples may have varied, preliminary screening of each sample prior to taste panel incorporation eliminated any samples with noticeable flavor defects.

Ballots were coded by assigning a value of 7.0 to the extreme left end of the line and a value of 1.0 to the extreme right end of the line. Each panelist's marked judgments were assigned an

FIGURE 1. BALLOT EMPLOYED FOR THE SENSORY EVALUATION OF STORAGE STABILITY SAMPLES

PRODUCT EVALUATION

NAME ______________________________ PRODUCT ____________________

JUDGE NUMBER ________________________ DATE ______________________

DIRECTIONS: Mark each line at the position that best expresses your description of each sample. Be sure to write the sample number above each mark.

A. OVERALL DESIRABILITY

1. Rate the Overall Desirability of each sample.

Extremely Acceptable |——————————————————————————| Extremely Unacceptable

B. OFF-FLAVOR

1. Rate the Intensity of the OXIDIZED flavor (if any).

EXTREME |——————————————————————————| NONE

2. Rate the intensity of any OTHER OFF-FLAVORS present (if any).

EXTREME |——————————————————————————| NONE

3. Describe any OFF-FLAVORS PRESENT, other than oxidized.

____________________ ____________________________

____________________ ____________________________

____________________ ____________________________

C. COMMENTS

appropriate numerical value (in 1.28 cm increments), and coded values were punched into IBM computer cards and analyzed on the University of Wisconsin 1110 Univac computer. Means were computed for each sample characteristic, and the F-value for the whole comparison and least significant differences (LSD's) at the 5% level of probability were computed for each pair of samples. Lines of best fit determined by linear and curvilinear regression analysis were computer drawn using a plotter program for the means of each food characteristic over the six-month storage period.

III. Results

The results of investigations employing radioactive antioxidants are given in Tables 3 through 34. Data relating to retention of radioactive antioxidants in products through processing after application by the various methods are presented in Tables 3 through 8. Tables 9 through 13 present the data for penetration and location of radioactive antioxidants in the frozen food systems. The data for the distribution of radioactive antioxidants into the various lipid and non-lipid fractions in foods are shown in Tables 14 through 19. The data showing the distribution and location of radioactive antioxidants within the frozen food systems are detailed in Tables 20 through 34.

The practical amounts of antioxidant necessary in each delivery system to achieve 200 ppm on a fat basis in the product is summarized in Table 35. Results of a storage study on rates of tritium exchange between ^{3}H-PG or ^{3}H-BHA and water are presented in Table 36. Tables 37 and 38 present data on the peroxidation of frying oil during extended periods at 204 C used for frying chicken legs and fish sticks, respectively. Data on retention of ^{14}C-BHT in frying oil held at 204 C is given in Table 39. Table 40 presents the results of chemical measurements of BHA in fish sticks prepared for the storage stability study.

The data relating to the storage stability of products prepared with and without combinations of unlabeled antioxidants are presented in Figures 2 through 43. Data used in preparing Figures 2 through 43 are presented in the appendix in Tables A 1 through A 18.

TABLE 3 RETENTION OF RADIOACTIVE ANTIOXIDANTS APPLIED BY VARIOUS METHODS TO CHICKEN LEGS

Methods of Application		Antioxidants				
		^{14}C-BHT	^{3}H-BHA	^{3}H-PG	^{14}C-CA	^{14}C-EDTA
		(% Retained[a])				
Direct Addition	During Mixing					
	With Salt Cure Mixture					
	During Chopping					
	With Ingredients					
Dipping	Before Cooking					
	After Cooking					
Spraying	Before Battering	103.5	30.7	38.9	89.6	86.7
	Before Freezing	100.0	100.0	100.0	100.0	100.0
With Batter		94.7	47.6	73.5	53.6	54.6
In Frying Oil		100.0	100.0			
Steam Blanching						
Immersion Freezing			100.0			
Freeze-Dry Vacuum Release						

[a] Percent of transferred antioxidant retained in food through processing.

TABLE 4 RETENTION OF RADIOACTIVE ANTIOXIDANTS APPLIED BY VARIOUS METHODS TO FISH STICKS

Methods of Application		Antioxidants				
		^{14}C-BHT	^{3}H-BHA	^{3}H-PG	^{14}C-CA	^{14}C-EDTA
		(% Retained[a])				
Direct Addition	During Mixing					
	With Salt Cure Mixture					
	During Chopping					
	With Ingredients					
Dipping	Before Cooking					
	After Cooking					
Spraying	Before Battering	101.6	61.3	35.0	51.0	65.9
	Before Freezing	100.0	100.0	100.0	100.0	100.0
With Batter		98.0	55.7	65.8	47.0	49.0
In Frying Oil		100.0	100.0			
Steam Blanching						
Immersion Freezing			100.0			
Freeze-Dry Vacuum Release						

[a] Percent of transferred antioxidant retained in food through processing.

TABLE 5 RETENTION OF RADIOACTIVE ANTIOXIDANTS APPLIED BY VARIOUS METHODS TO FRANKFURTERS

Methods of Application		Antioxidants ^{14}C-BHT	^{3}H-BHA	^{3}H-PG	^{14}C-CA	^{14}C-EDTA
		(% Retained[a])				
Direct Addition	During Mixing	98.8	91.1			
	With Salt Cure Mixture	95.5	101.5	95.5	79.9	84.9
	During Chopping	88.1	104.5	88.0	80.5	86.2
	With Ingredients					
Dipping	Before Cooking					
	After Cooking					
Spraying	Before Battering					
	Before Freezing					
With Batter						
In Frying Oil						
Steam Blanching						
Immersion Freezing			100.0			
Freeze-Dry Vacuum Release						

[a] Percent of transferred antioxidant retained in food through processing.

TABLE 6 RETENTION OF RADIOACTIVE ANTIOXIDANTS APPLIED BY VARIOUS METHODS TO PORK CHOPS

Methods of Application		Antioxidants: ^{14}C-BHT	^{3}H-BHA	^{3}H-PG	^{14}C-CA	^{14}C-EDTA
		(% Retained[a])				
Direct Addition	During Mixing					
	With Salt Cure Mixture					
	During Chopping					
	With Ingredients					
Dipping	Before Cooking	84.4	83.8	89.9	65.6	44.0
	After Cooking	86.6	82.3	74.0	85.3	91.6
Spraying	Before Battering					
	Before Freezing					
With Batter						
In Frying Oil						
Steam Blanching						
Immersion Freezing			95.5			
Freeze-Dry Vacuum Release			100.0			

[a] Percent of transferred antioxidant retained in food through processing.

TABLE 7 RETENTION OF RADIOACTIVE ANTIOXIDANTS APPLIED BY VARIOUS METHODS TO BEEF STEW

Methods of Application		Antioxidants ^{14}C-BHT	^{3}H-BHA	^{3}H-PG	^{14}C-CA	^{14}C-EDTA
		(% Retained[a])				
Direct Addition	During Mixing					
	With Salt Cure Mixture					
	During Chopping					
	With Ingredients	38.2	47.1	57.8	64.4	66.8
Dipping	Before Cooking	31.8	46.4	53.7	69.6	71.3
	After Cooking					
Spraying	Before Battering					
	Before Freezing					
With Batter						
In Frying Oil						
Steam Blanching						
Immersion Freezing			103.7			
Freeze-Dry Vacuum Release			100.0			

[a] Percent of transferred antioxidant retained in food through processing.

TABLE 8 RETENTION OF RADIOACTIVE ANTIOXIDANTS APPLIED BY VARIOUS METHODS TO CARROTS

Methods of Application		Antioxidants ^{14}C-BHT	^{3}H-BHA	^{3}H-PG	^{14}C-CA	^{14}C-EDTA
		(——— % Retained[a] ———				)
Direct Addition	During Mixing					
	With Salt Cure Mixture					
	During Chopping					
	With Ingredienta					
Dipping	Before Cooking					
	After Cooking					
Spraying	Before Battering					
	Before Freezing	21.4	44.6	67.0	102.0	81.1
With Batter						
In Frying Oil						
Steam Blanching			73.5			
Immersion Freezing		33.7	8.0			
Freeze-Dry Vacuum Release		100.0	100.0			

[a] Percent of transferred antioxidant retained in food through processing.

TABLE 9 PENETRATION AND LOCATION OF ^{14}C-BHT IN FROZEN FOOD SYSTEMS

Method of Application		Chicken Legs[a] Location 1	2	3	Fish Sticks[b] Location 1	2	3	Frankfurters[c] Location 1	2	3
		(% of Antioxidant Retained)								
Direct Addition	During Mixing	-	-	-	-	-	-	32.4	33.2	34.4
	With Salt Cure Mixture	-	-	-	-	-	-	35.5	37.3	27.2
	During Chopping	-	-	-	-	-	-	35.4	32.0	32.6
	With Ingredients	-	-	-	-	-	-	-	-	-
Dipping	Before Cooking	-	-	-	-	-	-	-	-	-
	After Cooking	-	-	-	-	-	-	-	-	-
Spraying	Before Battering	82.0	15.2	2.8	82.1	16.1	1.8	-	-	-
	Before Freezing	95.2	4.8	0.0	95.6	4.3	0.1	-	-	-
With Batter		87.6	9.8	2.6	71.8	26.6	1.6	-	-	-
In Frying Oil		96.3	3.4	0.3	93.7	5.5	0.8	-	-	-
Steam Blanching		-	-	-	-	-	-	-	-	-
Immersion Freezing		-	-	-	-	-	-	-	-	-
Freeze-Dry Vacuum Release		-	-	-	-	-	-	-	-	-

[a]Location: 1, Batter and breading; 2, skin; 3, muscle, per portion basis.

[b]Location: 1, Batter and breading; 2, outer layer; 3, center portion, per portion basis.

[c]Location: 1, Outer layer; 2, intermediate layer; 3, center portion, per gm basis (not diffusion--controlled distribution).

TABLE 10 PENETRATION AND LOCATION OF ^{3}H-BHA IN FROZEN FOOD SYSTEMS

Method of Application		Chicken Legs[a] Location 1	2	3	Fish Sticks[b] Location 1	2	3	Frankfurters[c] Location 1	2	3
		(% of Antioxidant Retained)								
Direct Addition	During Mixing	-	-	-	-	-	-	38.4	32.1	29.5
	With Salt Cure Mixture	-	-	-	-	-	-	38.3	31.4	30.3
	During Chopping	-	-	-	-	-	-	34.2	31.8	34.0
	With Ingredients	-	-	-	-	-	-	-	-	-
Dipping	Before Cooking	-	-	-	-	-	-	-	-	-
	After Cooking	-	-	-	-	-	-	-	-	-
Spraying	Before Battering	64.1	33.7	2.2	68.2	26.8	5.0	-	-	-
	Before Freezing	88.5	9.7	1.8	97.2	2.4	0.4	-	-	-
With Batter		77.1	12.8	10.1	75.2	17.4	7.4	-	-	-
In Frying Oil		86.6	13.0	0.4	93.1	6.4	0.5	-	-	-
Steam Blanching		-	-	-	-	-	-	-	-	-
Immersion Freezing		33.5	7.9	58.6	95.0	4.3	0.7	53.4	13.8	32.8
Freeze-Dry Vacuum Release		-	-	-	-	-	-	-	-	-

[a]Location: 1, Batter and breading; 2, skin; 3, muscle, per portion basis.

[b]Location: 1, Batter and breading; 2, outer layer; 3, center portion, per portion basis.

[c]Location: 1, Outer layer; 2, intermediate layer; 3, center portion, per gm basis (not diffusion--controlled distribution).

TABLE 11 PENETRATION AND LOCATION OF ^{3}H-PG IN FROZEN FOOD SYSTEMS

Method of Application		Chicken Legs[a] Location 1	2	3	Fish Sticks[b] Location 1	2	3	Frankfurters[c] Location 1	2	3
		(— % of Antioxidant Retained —)								
Direct Addition	During Mixing	-	-	-	-	-	-	-	-	-
	With Salt Cure Mixture	-	-	-	-	-	-	35.3	29.4	35.3
	During Chopping	-	-	-	-	-	-	36.9	32.6	30.5
	With Ingredients	-	-	-	-	-	-	-	-	-
Dipping	Before Cooking	-	-	-	-	-	-	-	-	-
	After Cooking	-	-	-	-	-	-	-	-	-
Spraying	Before Battering	32.4	58.1	9.5	66.6	22.2	11.1	-	-	-
	Before Freezing	89.1	5.7	5.2	92.9	4.4	2.7	-	-	-
With Batter		83.7	11.0	5.3	82.2	14.6	3.2	-	-	-
In Frying Oil		-	-	-	-	-	-	-	-	-
Steam Blanching		-	-	-	-	-	-	-	-	-
Immersion Freezing		-	-	-	-	-	-	-	-	-
Freeze-Dry Vacuum Release		-	-	-	-	-	-	-	-	-

[a]Location: 1, Batter and breading; 2, skin; 3, muscle, per portion basis.

[b]Location: 1, Batter and breading; 2, outer layer; 3, center portion, per portion basis.

[c]Location: 1, Outer layer; 2, intermediate layer; 3, center portion, per gm basis (not diffusion--controlled distribution).

TABLE 12 PENETRATION AND LOCATION OF ^{14}C-CA IN FROZEN FOOD SYSTEMS

Method of Application		Chicken Legs[a] Location 1	2	3	Fish Sticks[b] Location 1	2	3	Frankfurters[c] Location 1	2	3
		(% of Antioxidant Retained)								
Direct Addition	During Mixing	-	-	-	-	-	-	-	-	-
	With Salt Cure Mixture	-	-	-	-	-	-	30.8	34.3	34.9
	During Chopping	-	-	-	-	-	-	24.8	34.2	41.0
	With Ingredients	-	-	-	-	-	-	-	-	-
Dipping	Before Cooking	-	-	-	-	-	-	-	-	-
	After Cooking	-	-	-	-	-	-	-	-	-
Spraying	Before Battering	52.9	22.4	24.7	48.2	32.2	19.6	-	-	-
	Before Freezing	98.3	1.7	0.0	76.0	16.7	7.3	-	-	-
With Batter		58.5	20.4	21.1	58.9	32.2	8.9	-	-	-
In Frying Oil		-	-	-	-	-	-	-	-	-
Steam Blanching		-	-	-	-	-	-	-	-	-
Immersion Freezing		-	-	-	-	-	-	-	-	-
Freeze-Dry Vacuum Release		-	-	-	-	-	-	-	-	-

[a]Location: 1, Batter and breading; 2, skin; 3, muscle, per portion basis.

[b]Location: 1, Batter and breading; 2, outer layer; 3, center portion, per portion basis.

[c]Location: 1, Outer layer; 2, intermediate layer; 3, center portion, per gm basis (not diffusion--controlled distribution).

TABLE 13 PENETRATION AND LOCATION OF ^{14}C-EDTA IN FROZEN FOOD SYSTEMS

Method of Application		Chicken Legs[a] Location 1	2	3	Fish Sticks[b] Location 1	2	3	Frankfurters[c] Location 1	2	3
		(——— % of Antioxidant Retained ———)								
Direct Addition	During Mixing	-	-	-	-	-	-	-	-	-
	With Salt Cure Mixture	-	-	-	-	-	-	29.7	36.9	33.4
	During Chopping	-	-	-	-	-	-	39.4	31.1	29.5
	With Ingredients	-	-	-	-	-	-	-	-	-
Dipping	Before Cooking	-	-	-	-	-	-	-	-	-
	After Cooking	-	-	-	-	-	-	-	-	-
Spraying	Before Battering	61.9	24.8	13.3	44.8	32.8	22.4	-	-	-
	Before Freezing	98.4	1.3	0.3	97.2	2.2	0.6	-	-	-
With Batter		61.7	22.9	15.4	66.1	27.0	6.9	-	-	-
In Frying Oil		-	-	-	-	-	-	-	-	-
Steam Blanching		-	-	-	-	-	-	-	-	-
Immersion Freezing		-	-	-	-	-	-	-	-	-
Freeze-Dry Vacuum Release		-	-	-	-	-	-	-	-	-

[a]Location: 1, Batter and breading; 2, skin; 3, muscle, per portion basis.

[b]Location: 1, Batter and breading; 2, outer layer; 3, center portion, per portion basis.

[c]Location: 1, Outer layer; 2, intermediate layer; 3, center portion, per gm basis (not diffusion--controlled distribution).

TABLE 14 DISTRIBUTION OF RADIOACTIVE ANTIOXIDANTS IN VARIOUS FRACTIONS OF CHICKEN LEGS

Methods of Application		ANTIOXIDANT																			
		^{14}C-BHT Fraction[a]				^{3}H-BHA Fraction				^{3}H-PG Fraction				^{14}C-CA Fraction				^{14}C-EDTA Fraction			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
		(% of Total)																			
Direct Addition	During Mixing With Salt Cure Mixture																				
	During Chopping With Ingredients																				
Dipping	Before Cooking																				
	After Cooking																				
Spraying	Before Battering	44.0	46.8	8.1	7.1	93.1	2.5	2.8	1.6	31.6	14.9	53.5	0	0	0	80.0	20.0	0	0	70.9	29.1
	Before Freezing	94.4	4.7	0.9	0	97.0	0.5	1.[illegible]	0.9	29.0	33.9	23.3	13.8	0	1.4	65.6	33.0	0	0.5	75.4	24.1
With Batter		48.0	38.9	5.6	7.5	59.9	15.3	11.6	13.2	11.2	44.0	31.0	13.9	0	0	78.3	21.7	0	0	75.5	24.5
In Frying Oil		93.5	5.6	0.8	0.1	96.8	1.8	1.4	0												
Steam Blanching																					
Immersion Freezing						53.1	11.9	31.9	2.1												
Freeze-Dry Vacuum Release																					

[a] 1 = Free Lipid, 2 = Bound Lipid, 3 = Aqueous, 4 = Solid Fraction

TABLE 15 DISTRIBUTION OF RADIOACTIVE ANTIOXIDANTS IN VARIOUS FRACTIONS OF FISH STICKS

Methods of Application		ANTIOXIDANT																			
		^{14}C-BHT Fraction[a]				^{3}H-BHA Fraction				^{3}H-PG Fraction				^{14}C-CA Fraction				^{14}C-EDTA Fraction			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
		(% of Total)																			
Direct Addition	During Mixing																				
	With Salt Cure Mixture																				
	During Chopping																				
	With Ingredients																				
Dipping	Before Cooking																				
	After Cooking																				
Spraying	Before Battering	59.2	38.7	1.6	0.5	87.3	8.1	3.7	0.9	9.5	18.2	42.3	30.0	0	0	84.9	15.1	0	0	93.2	6.8
	Before Freezing	93.8	5.1	1.1	0	96.2	1.7	1.5	0.6	13.5	26.4	28.4	31.7	0	0	95.3	4.7	0	0	90.6	9.4
With Batter		56.2	39.0	2.2	2.6	66.9	10.5	9.8	12.8	6.3	34.1	24.3	35.3	0	0	87.9	12.1	0	0	77.7	22.3
In Frying Oil		94.3	4.5	0.7	0.5	96.4	0.6	2.2	0.8												
Steam Blanching																					
Immersion Freezing						91.8	5.4	2.5	0.3												
Freeze-Dry Vacuum Release																					

[a] 1 = Free Lipid, 2 = Bound Lipid, 3 = Aqueous, 4 = Solid Fraction

TABLE 16 DISTRIBUTION OF RADIOACTIVE ANTIOXIDANTS IN VARIOUS FRACTIONS OF FRANKFURTERS

Methods of Application		ANTIOXIDANT																			
		^{14}C-BHT Fraction[a]				^{3}H-BHA Fraction				^{3}H-PG Fraction				^{14}C-CA Fraction				^{14}C-EDTA Fraction			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
		(——— % of Total ———)																			
Direct Addition	During Mixing	43.9	44.2	0	11.8	51.7	25.5	2.0	20.8												
	With Salt Cure Mixture	47.6	39.7	0	12.7	75.0	20.6	1.2	3.1	12.6	22.7	31.8	32.9	0	0	73.2	26.8	0	0	71.8	28.2
	During Chopping	49.5	38.2	0	12.3	79.1	16.1	1.5	3.3	13.5	18.0	35.7	32.8	0	0	61.4	38.6	0	0	69.9	30.1
	With Ingredients																				
Dipping	Before Cooking																				
	After Cooking																				
Spraying	Before Battering																				
	Before Freezing																				
With Batter																					
In Frying Oil																					
Steam Blanching																					
Immersion Freezing						23.0	4.7	66.4	5.9												
Freeze-Dry Vacuum Release																					

[a] 1 = Free Lipid, 2 = Bound Lipid, 3 = Aqueous, 4 = Solid Fraction

TABLE 17 DISTRIBUTION OF RADIOACTIVE ANTIOXIDANTS IN VARIOUS FRACTIONS OF PORK CHOPS

Methods of Application		ANTIOXIDANT ^{14}C-BHT Fraction[a] 1	2	3	4	^{3}H-BHA Fraction 1	2	3	4	^{3}H-PG Fraction 1	2	3	4	^{14}C-CA Fraction 1	2	3	4	^{14}C-EDTA Fraction 1	2	3	4
		(% of Total)																			
Direct Addition	During Mixing With Salt Cure Mixture																				
	During Chopping																				
	With Ingredients																				
Dipping	Before Cooking	42.2	48.9	7.7	1.2	75.1	3.0	18.0	3.9	1.1	45.5	47.5	5.9	1.4	0	50.3	48.3	0.5	1.4	55.6	42.5
	After Cooking	63.1	27.1	8.4	1.4	75.7	2.4	18.2	3.7	2.2	46.8	44.4	6.6	0.6	0	45.7	53.7	0.7	0	72.8	26.5
Spraying	Before Battering																				
	Before Freezing																				
With Batter																					
In Frying Oil																					
Steam Blanching																					
Immersion Freezing						59.8	30.9	8.5	0.8												
Freeze-Dry Vacuum Release						78.9	5.9	11.7	3.5												

[a] 1 = Free Lipid, 2 = Bound Lipid, 3 = Aqueous, 4 = Solid Fraction

TABLE 18 DISTRIBUTION OF RADIOACTIVE ANTIOXIDANTS IN VARIOUS FRACTIONS OF BEEF STEW

Methods of Application		ANTIOXIDANT																			
		^{14}C-BHT Fraction[e]				^{3}H-BHA Fraction				^{3}H-PG Fraction				^{14}C-CA Fraction				^{14}C-EDTA Fraction			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
		(% of Total)																			
Direct Addition	During Mixing																				
	With Salt Cure Mixture																				
	During Chopping																				
	With Ingredients	79.3	10.0	1.3	9.4	84.1	10.0	5.5	0.4	20.6	20.9	56.9	1.6	0	0	85.2	14.8	0	0	90.4	9.6
Dipping	Before Cooking	90.0	9.1	0.6	0.3	32.1	59.2	7.8	0.9	16.0	37.2	40.0	6.8	0	0	94.1	5.9	0	0	91.7	8.3
	After Cooking																				
Spraying	Before Battering																				
	Before Freezing																				
With Batter																					
In Frying Oil																					
Steam Blanching																					
Immersion Freezing						71.5	2.0	26.5	0												
Freeze-Dry Vacuum Release																					

[e] 1 = Free Lipid, 2 = Bound Lipid, 3 = Aqueous, 4 = Solid Fraction

TABLE 19 DISTRIBUTION OF RADIOACTIVE ANTIOXIDANTS IN VARIOUS FRACTIONS OF CARROTS

Methods of Application		^{14}C-BHT Fraction[a] 1	2	3	4	^{3}H-BHA Fraction 1	2	3	4	^{3}H-PG Fraction 1	2	3	4	^{14}C-CA Fraction 1	2	3	4	^{14}C-EDTA Fraction 1	2	3	4
		(% of Total)																			
Direct Addition	During Mixing																				
	With Salt Cure Mixture																				
	During Chopping																				
	With Ingredients																				
Dipping	Before Cooking																				
	After Cooking																				
Spraying	Before Battering																				
	Before Freezing	58.4	36.5	1.4	3.7	89.9	7.2	1.1	1.8	17.6	34.3	46.3	1.9	0.7	0	63.7	35.8	0	0	65.0	35.0
With Batter																					
In Frying Oil																					
Steam Blanching						66.1	8.3	24.6	1.0												
Immersion Freezing		97.9	0.9	0.3	0.9	60.0	20.0	20.0	0												
Freeze-Dry Vacuum Release		96.8	1.5	1.0	0.7	94.0	0	5.8	0.2												

[a] 1 = Free Lipid, 2 = Bound Lipid, 3 = Aqueous, 4 = Solid Fraction

TABLE 20 DISTRIBUTION OF ^{14}C-BHT ACCORDING TO LOCATION IN BREADED CHICKEN LEGS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction: Free lipid	Bound lipid	Aqueous non-lipid	Solid non-lipid
		(——— % of Total			———)
Spraying Before Battering	Batter and Bread	38.7	35.4	2.6	5.3
	Skin	4.2	5.21	4.6	1.1
	Muscle	1.1	0.19	0.9	0.7
With Batter	Batter and Bread	42.0	36.2	3.5	6.0
	Skin	4.8	2.4	1.3	1.3
	Muscle	1.2	0.3	0.8	0.2
Spraying Before Freezing	Batter and Bread	89.6	4.7	0.9	0.0
	Skin	4.8	0.0	0.0	0.0
	Muscle	0.0	0.0	0.0	0.0
In Frying Oil	Batter and Bread	90.0	5.4	0.8	0.1
	Skin	3.2	0.2	0.0	0.0
	Muscle	0.3	0.0	0.0	0.0

TABLE 21 DISTRIBUTION OF ^{3}H-BHA ACCORDING TO LOCATION IN BREADED CHICKEN LEGS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction			
		Free lipid	Bound lipid	Aqueous non-lipid	Solid non-lipid
		(——— % of Total ———)			
Spraying Before Battering	Batter and Bread	60.3	1.6	1.6	0.6
	Skin	30.6	.9	1.3	0.9
	Muscle	2.2	0.0	0.0	0.0
With Batter	Batter and Bread	49.4	12.8	3.8	11.1
	Skin	8.2	1.7	2.1	8.4
	Muscle	2.3	.8	5.7	1.3
Spraying Before Freezing	Batter and Bread	86.7	0.5	0.4	0.9
	Skin	9.5	0.0	0.2	0.0
	Muscle	0.8	0.0	1.0	0.0
In Frying Oil	Batter and Bread	83.6	1.8	1.2	0.0
	Skin	12.8	0.0	0.2	0.0
	Muscle	0.4	0.0	0.0	0.0
Immersion Freezing	Batter and Bread	23.2	3.4	6.0	0.9
	Skin	3.9	1.4	2.1	0.5
	Muscle	26.0	7.1	23.8	1.7

TABLE 22 DISTRIBUTION OF ^{3}H-PG ACCORDING TO LOCATION IN BREADED CHICKEN LEGS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction: Free lipid	Bound lipid	Aqueous non-lipid	Solid non-lipid
		(% of Total)			
Spraying Before Battering	Batter and Bread	14.4	3.9	14.1	0.0
	Skin	13.6	9.0	35.5	0.0
	Muscle	3.6	2.0	3.9	0.0
With Batter	Batter and Bread	9.3	36.6	26.2	11.7
	Skin	1.5	5.2	2.3	2.0
	Muscle	0.4	2.2	2.6	0.1
Spraying Before Freezing	Batter and Bread	25.3	32.9	19.2	11.7
	Skin	2.2	0.7	1.7	1.1
	Muscle	1.5	0.3	2.4	1.0

TABLE 23 DISTRIBUTION OF ^{14}C-CA ACCORDING TO LOCATION IN BREADED CHICKEN LEGS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction: Free lipid	Bound lipid	Aqueous non-lipid	Solid non-lipid
		(% of Total)			
Spraying Before Battering	Batter and Bread	0.0	0.0	42.1	10.7
	Skin	0.0	0.0	16.9	5.6
	Muscle	0.0	0.0	21.0	3.7
With Batter	Batter and Bread	0.0	0.0	48.0	10.5
	Skin	0.0	0.0	11.0	9.4
	Muscle	0.0	0.0	19.3	1.8
Spraying Before Freezing	Batter and Bread	0.0	1.4	64.2	32.7
	Skin	0.0	0.0	1.4	0.3
	Muscle	0.0	0.0	0.0	0.0

TABLE 24 DISTRIBUTION OF ^{14}C-EDTA ACCORDING TO LOCATION IN BREADED CHICKEN LEGS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction			
		Free lipid	Bound lipid	Aqueous non-lipid	Solid non-lipid
		(% of Total)			
Spraying Before Battering	Batter and Bread	0.0	0.0	43.1	18.8
	Skin	0.0	0.0	16.3	8.6
	Muscle	0.0	0.0	11.5	1.7
With Batter	Batter and Bread	0.0	0.0	47.8	13.9
	Skin	0.0	0.0	13.6	9.3
	Muscle	0.0	0.0	14.1	1.3
Spraying Before Freezing	Batter and Bread	0.0	0.5	73.9	24.0
	Skin	0.0	0.0	1.2	0.1
	Muscle	0.0	0.0	0.3	0.0

TABLE 25 DISTRIBUTION OF ^{14}C-BHT ACCORDING TO LOCATION IN FISH STICKS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction			
		Free lipid	Bound lipid	Aqueous non-lipid	Solid non-lipid
		(% of Total)			
Spraying Before Battering	Batter and Bread	51.4	29.6	0.5	0.4
	Outer	7.0	8.8	0.3	0.1
	Center	0.8	0.3	0.7	0.0
With Batter	Batter and Bread	41.0	27.3	2.0	1.5
	Outer	14.4	11.7	0.2	0.3
	Center	0.8	0.0	0.0	0.8
Spraying Before Freezing	Batter and Bread	89.6	4.9	1.1	0.0
	Outer	4.1	0.2	0.0	0.0
	Center	0.1	0.0	0.0	0.0
In Frying Oil	Batter and Bread	89.5	3.7	0.2	0.3
	Outer	4.5	0.6	0.2	0.2
	Center	0.3	0.2	0.3	0.2

TABLE 26 DISTRIBUTION OF ^{3}H-BHA ACCORDING TO LOCATION IN FISH STICKS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction: Free lipid	Bound lipid	Aqueous non-lipid	Solid non-lipid
		(% of Total)			
Spraying Before Battering	Batter and Bread	61.9	3.8	2.1	0.5
	Outer	22.8	2.8	0.8	0.3
	Center	2.61	1.5	0.8	0.1
With Batter	Batter and Bread	58.1	3.7	5.0	8.2
	Outer	7.9	1.6	4.8	3.4
	Center	0.9	5.2	0.0	1.2
In Frying Oil	Batter and Bread	90.5	0.6	1.3	0.7
	Outer	5.4	0.0	0.9	0.1
	Center	0.5	0.0	0.0	0.0
Spraying Before Freezing	Batter and Bread	94.1	1.7	1.0	0.4
	Outer	2.0	0.0	0.3	0.1
	Center	0.1	0.0	0.2	0.1
Immersion Freezing	Batter and Bread	88.6	4.6	1.7	0.1
	Outer	2.9	0.5	0.8	0.1
	Center	0.3	0.3	0.0	0.1

TABLE 27 DISTRIBUTION OF ^{3}H-PG ACCORDING TO LOCATION IN FISH STICKS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction			
		Free lipid	Bound lipid	Aqueous non-lipid	Solid non-lipid
		(% of Total)			
Spraying Before Battering	Batter and Bread	3.6	13.1	24.6	20.3
	Outer	0.9	3.1	11.1	7.1
	Center	0.0	2.0	6.6	2.6
With Batter	Batter and Bread	5.2	30.8	16.1	30.1
	Outer	0.8	3.0	6.2	4.6
	Center	0.3	0.3	2.0	0.6
Spraying Before Freezing	Batter and Bread	12.9	25.4	23.5	31.1
	Outer	0.3	0.8	3.0	0.3
	Center	0.3	0.2	1.9	0.3

TABLE 28 DISTRIBUTION OF ^{14}C-CA ACCORDING TO LOCATION IN FISH STICKS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction			
		Free lipid	Bound lipid	Aqueous non-lipid	Solid non-lipid
		(% of Total)			
Spraying Before Battering	Batter and Bread	0.0	0.0	40.6	7 6
	Outer	0.0	0.0	27.0	5.1
	Center	0.0	0.0	17.3	2.4
With Batter	Batter and Bread	0.0	0.0	49.4	9.6
	Outer	0.0	0.0	29.8	2.3
	Center	0.0	0.0	8.7	0.2
Spraying Before Freezing	Batter and Bread	0.0	0.0	73.0	3.0
	Outer	0.0	0.0	15.3	1.4
	Center	0.0	0.0	7.0	0.3

TABLE 29 DISTRIBUTION OF ^{14}C-EDTA ACCORDING TO LOCATION IN FISH STICKS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction			
		Free lipid	Bound lipid	Aqueous non-lipid	Solid non-lipid
		(% of Total)			
Spraying Before Battering	Batter and Bread	0.0	0.0	41.3	3.6
	Outer	0.0	0.0	30.9	1.8
	Center	0.0	0.0	21.0	1.4
With Batter	Batter and Bread	0.0	0.0	49.6	16.6
	Outer	0.0	0.0	21.6	5.3
	Center	0.0	0.0	6.5	0.4
Spraying Before Freezing	Batter and Bread	0.0	0.0	87.8	9.4
	Outer	0.0	0.0	2.2	0.0
	Center	0.0	0.0	0.6	0.0

TABLE 30 DISTRIBUTION OF ^{14}C-BHT ACCORDING TO LOCATION IN FRANKFURTERS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction: Free lipid	Fraction: Bound lipid	Fraction: Aqueous non-lipid	Fraction: Solid non-lipid
		(——— % of Total			———)
Direct Addition During Mixing	Outer	14.0	14.0	0.0	4.4
	Intermediate	14.3	15.3	0.0	3.6
	Center	15.6	14.9	0.0	3.8
Direct Addition With Salt Cure Mixture	Outer	15.1	14.1	0.0	6.3
	Intermediate	20.6	13.2	0.0	3.5
	Center	11.9	12.4	0.0	2.9
Direct Addition During Chopping	Outer	18.0	11.8	0.0	5.6
	Intermediate	15.3	13.2	0.0	3.5
	Center	16.2	13.2	0.0	3.2

TABLE 31 DISTRIBUTION OF ^{3}H-BHA ACCORDING TO LOCATION IN FRANKFURTERS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction			
		Free lipid	Bound lipid	Aqueous non-lipid	Solid non-lipid
		(% of Total)			
Direct Addition During Mixing	Outer	17.8	10.8	0.7	9.1
	Intermediate	18.8	5.7	0.9	6.7
	Center	15.1	9.0	0.4	5.0
Direct Addition With Salt Cure Mixture	Outer	25.3	10.8	0.4	1.8
	Intermediate	26.0	4.4	0.4	0.6
	Center	23.8	5.4	0.4	0.7
Direct Addition During Chopping	Outer	24.8	6.9	0.5	2.0
	Intermediate	25.9	4.7	0.5	0.7
	Center	28.4	4.5	0.5	0.6
Immersion Freezing	Outer	16.1	0.0	34.6	2.7
	Intermediate	0.0	0.0	12.3	1.5
	Center	6.9	4.7	19.5	1.7

TABLE 32 DISTRIBUTION OF ^{3}H-PG ACCORDING TO LOCATION IN FRANKFURTERS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction			
		Free lipid	Bound lipid	Aqueous non-lipid	Solid non-lipid
		(——— % of Total			———)
Direct Addition With Salt Cure Mixture	Outer	4.3	6.4	10.8	13.8
	Intermediate	4.2	8.0	9.8	7.4
	Center	4.1	8.3	11.2	11.7
Direct Addition During Chopping	Outer	5.0	6.1	12.3	13.5
	Intermediate	4.3	5.5	11.9	10.9
	Center	4.2	6.4	11.5	8.4

TABLE 33 DISTRIBUTION OF ^{14}C-CA ACCORDING TO LOCATION IN FRANKFURTERS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction			
		Free lipid	Bound lipid	Aqueous non-lipid	Solid non-lipid
		(% of Total)			
Direct Addition With Salt Cure Mixture	Outer	0.0	0.0	20.4	10.4
	Intermediate	0.0	0.0	26.3	8.0
	Center	0.0	0.0	26.5	8.4
Direct Addition During Chopping	Outer	0.0	0.0	15.8	9.1
	Intermediate	0.0	0.0	22.7	11.4
	Center	0.0	0.0	22.9	18.1

TABLE 34 DISTRIBUTION OF ^{14}C-EDTA ACCORDING TO LOCATION IN FRANKFURTERS FOLLOWING DIFFERENT APPLICATION METHODS

Application Method	Location of Portion	Fraction: Free lipid	Bound lipid	Aqueous non-lipid	Solid non-lipid
		(% of Total)			
Direct Addition With Salt Cure Mixture	Outer	0.0	0.0	19.2	10.5
	Intermediate	0.0	0.0	29.1	7.8
	Center	0.0	0.0	23.5	9.9
Direct Addition During Chopping	Outer	0.0	0.0	24.4	15.1
	Intermediate	0.0	0.0	23.7	7.4
	Center	0.0	0.0	21.8	7.6

TABLE 35 AMOUNT OF ANTIOXIDANTS IN DELIVERY SYSTEMS TO ACHIEVE 200 PPM (FAT BASIS) IN FINISHED PRODUCTS

Method of Application	Antioxidant	Concentration of Antioxidant: Experimental level in food product (µg/g fat)	Concentration of Antioxidant: Amount in delivery system to achieve 200 ppm, fat basis	Amount of Carrier
Chicken Legs				
Spraying Before Battering	CA	180	2.1% of CA in propylene glycol	Ca. 0.052 ml propylene glycol or 0.066 ml corn oil per chicken leg.
	EDTA	174	2.2% of EDTA in propylene glycol	
	BHT	200	1.5% of BHT in corn oil	
	BHA	62	4.9% of BHA in corn oil	
	PG	78	3.9% of PG in corn oil	
Spraying Before Freezing	CA	200	1.9% of CA or EDTA in propylene glycol	Ca. 0.052 ml propylene glycol or 0.066 ml corn oil for chicken leg.
	EDTA	200		
	BHT	200		
	BHA	200	1.5% of BHT, BHA or PG in corn oil	
	PG	200		
With Batter	CA	112	277 (ug/g of batter)	Ca. 6.4 g batter per chicken leg.
	EDTA	110	286	
	BHT	190	165	
	BHA	96	327	
	PG	78	212	
In Frying Oil	BHT BHA	200	754 ug/ml of frying oil	Ca. 5.48 ml frying oil absorbed per chicken leg.
Immersion Freezing	BHA	200	1.2 mg BHA/ml of Freon	Ca. 6.4 ml Freon-oil mixture per chicken leg.

Table 35 - Continued

Method of Application	Antioxidant	Concentration of Antioxidant: Experimental level in food product (μg/g fat)	Concentration of Antioxidant: Amount in delivery system to achieve 200 ppm, fat basis	Amount of Carrier
Fish Sticks				
Spraying Before Battering	CA	103	5.2% CA in propylene glytol	Ca. 0.019 ml propylene glycol or 0.024 ml corn oil per fish stick.
	EDTA	132	4.0% EDTA in propylene glycol	
	BHT	200	1.8% BHT in corn oil	
	BHA	122	3.0% BHA in corn oil	
	PG	70	5.1% PG in corn oil	
With Batter	CA	94	151 (ug/g of batter)	Ca. 6.5 g batter per fish stick.
	EDTA	9B	145	
	BHT	196	72	
	BHA	112	127	
	PG	132	10B	
Spraying Before Freezing	CA	200	1.9% of CA or EDTA in propylene glycol	Ca. 0.026 ml propylene glycol or 0.033 ml corn oil.
	EDTA	200		
	BHT	200		
	BHA	200	1.5% of BHA, BHT or PG in corn oil	
	PG	200		
In Frying Oil	BHT BHA	200	200 ug/g of frying oil	Ca. 2.75 ml frying oil absorbed per fish stick.
Immersion Freezing	BHA	200	65.3 ug BHA/ml of Freon	Ca. 7.6 ml Freon-oil mixture per fish stick.

Table 35 - Continued

Method of Application	Antioxidant	Concentration of Antioxidant: Experimental level in food product (μg/g fat)	Concentration of Antioxidant: Amount in delivery system to achieve 200 ppm, fat basis	Amount of Carrier
<u>Frankfurters</u>				
During Mixing	BHT	198	51 ug antioxidant/g frankfurter	--
	BHA	182	55 " "	
With Salt Cure Mixture	CA	160	62 " "	--
	EDTA	170	59 " "	
	BHT	192	53 " "	
	BHA	200	50 " "	
	PG	192	52 " "	
During Chopping	CA	162	62 " "	--
	EDTA	172	58 " "	
	BHT	176	57 " "	
	BHA	200	50 " "	
	PG	176	57 " "	
Immersion Freezing	BHA	200	1.5 mg BHA/ml Freon	Ca. 1.6 ml Freon-oil mixture per frankfurter
<u>Pork Chops</u>				
Dipping Before Cooking	CA	132	0.02% of CA in propylene glycol	Ca. 8.4 ml corn oil or 7.0 ml propylene glycol adsorbed per 100 g pork chop.
	EDTA	88	0.03% of EDTA in propylene glycol	
	BHT	168	0.02% of BHT in corn oil	
	BHA	166	0.02% of BHA in corn oil	
	PG	180	0.02% of PG in corn oil	

Table 35 - Continued

Method of Application	Antioxidant	Concentration of Antioxidant: Experimental level in food product (µg/g fat)	Concentration of Antioxidant: Amount in delivery system to achieve 200 ppm, fat basis	Amount of Carrier
Pork Chops (Continued)				
	CA	170	0.02% of CA in propylene glycol	Ca. 8.4 ml corn oil or 7.0 ml propylene glycol adsorbed per 100 g pork chop.
Dipping	EDTA	184	0.05% of EDTA in propylene glycol	
Before	BHT	174	0.02% of BHT in corn oil	
Cooking	BHA	164	0.02% of BHT in corn oil	
	PG	148	0.02% of BHT in corn oil	
Immersion Freezing	BHA	192	215 ug BHA/ml Freon	Ca. 6.7 ml Freon-oil mixture per 100 g pork chops.
Freeze-Dry Vacuum Release	BHA@100°C	Replicate 1 210 2 70 3 180 4 53 Ave. 128	0.39 g BHA in chamber, 20 g product	--
Beef Stew				
	CA	140	37i ug CA/ml propylene glycol	1 ml propylene glycol or corn oil added per 100 gm beef stew.
Direct Addition	EDTA	142	366 ug EDTA/ml propylene glycol	
With	BHT	64	812 ug BHT/ml corn oil	
Ingredients	BHA	92	565 ug BHA/ml corn oil	
	PG	108	481 ug PG/ml corn oil	

Table 35 - Continued

Method of Application	Antioxidant	Concentration of Antioxidant: Experimental level in food product (μg/g fat)	Concentration of Antioxidant: Amount in delivery system to achieve 200 ppm, fat basis	Amount of Carrier
Beef Stew (Continued)				
	CA	130	0.005% of CA in propylene glycol	Ca. 4.0 ml corn oil or 2.0 ml propylene glycol adsorbed per 100 g beef stew.
Dipping	EDTA	134	0.005% of EDTA in propylene glycol	
Before	BHT	76	0.017% of BHT in corn oil	
Cooking	BHA	94	0.014% of BHA in corn oil	
	PG	116	0.011% of PG in corn oil	
Immersion Freezing	BHA	200	104 ug BHA/ml Frecn	Ca. 2.5 ml Freon-oil mixture per 100 g beef stew.
Freeze-Dry Vacuum Release	BHA@100 C	Replicate 1 93 2 7 Ave. 50	1.0 g of BHA in chamber, 100 g product	--
Carrots				
	CA	200	0.073% CA in propylene glycol	Ca. 0.12 ml corn oil or 0.31 ml propylene glycol per 100 g carrot dice.
Spraying	EDTA	162	0.090% EDTA in propylene glycol	
Before	BHT	42	0.135% of BHT in corn oil	
Freezing	BHA	90	0.063% of BHA in corn oil	
	PG	134	0.042% of PG in corn oil	

Table 35 - Continued

Method of Application	Antioxidant	Concentration of Antioxidant: Experimental level in food product (µg/g fat)	Concentration of Antioxidant: Amount in delivery system to achieve 200 ppm, fat basis	Amount of Carrier
Carrots (Continued)				
Steam Blanching	BHA	2.8×10^6	19.2 ug of BHA in chamber	--
Immersion	BHT	68	15 ug BHT/ml Freon	Ca. 6.7 ml Freon-oil mixture per 10C g carrots.
Freezing	BHA	16	64 ug BHA/ml Freon	
	BHA @17 C	4	0.11 g of BHA at 100 C in chamber, 15 g product	--
	BHA @50 C	180		
	BHA @100 C	Replicate 1 550 2 382 Ave. 466		
	BHT @50 C	100	0.014 g of BHT at 100 C in chamber, 15 g product	--
	BHT @100 C	Replicate 1 5000 2 2140 Ave. 3570		

TABLE 36 TRITIUM DISTRIBUTION BETWEEN WATER AND ETHYLACETATE FROM ^{3}H-PG AND ^{3}H-BHA[a] AT 5 C[a]

Storage Time (wk)	^{3}H-PG			^{3}H-BHA		
	pH 5	pH 6	pH 7	pH 5	pH 6	pH 7
	(——— % radioactivity in ethylacetate phase ———)					
Initial	80.0	81.8	75.0	96.8	98.0	96.7
1	78.7	77.3	62.9	96.0	95.5	93.8
3	75.0	65.5	54.5	95.6	92.9	88.6
5	75.0	64.3	52.4	93.8	83.3	83.3
7	71.4	64.3	47.4	89.8	77.3	77.8
9	67.7	62.9	37.5	88.2	70.6	78.3

[a]Sample was stored at 5 C.

TABLE 37 PEROXIDE VALUES OF COTTONSEED FRYING OIL DURING FRYING OPERATION FOR CHICKEN LEGS

Time in Fryer at 204 C (min)	Peroxide value of frying oil (meq/kg)	
	WO BHT/EDTA	W BHT/EDTA
10	0.42 ± 0.08	0.72 ± 0.06
20	0.53 ± 0.14	0.96 ± 0.03
30	0.52 ± 0.12	1.03 ± 0.13
40	0.45 ± 0.07	1.02 ± 0.24
50	1.16 ± 0.27	0.92 ± 0.25
60	0.99 ± 0.22	0.83 ± 0.11
70	1.07 ± 0.22	0.94 ± 0.11
80	0.98 ± 0.24	1.19 ± 0.31
90	0.90 ± 0.35	1.16 ± 0.33

TABLE 38 PEROXIDE VALUES OF COTTONSEED (FRYING) OIL DURING FRYING OPERATION FOR FISH STICKS

Time in Fryer at 204 C (min)	Peroxide value of frying oil (meq/kg)	
	WO BHA/CA	W BHA/CA
15	2.08 ± 0.08	1.77 ± 0.06
30	1.99 ± 0.08	1.90 ± 0.03
45	2.00 ± 0.16	2.02 ± 0.14
60	1.80 ± 0.13	2.16 ± 0.08
75	2.00 ± 0.11	2.50 ± 0.27
90	1.88 ± 0.22	2.19 ± 0.31
105	2.19 ± 0.14	2.38 ± 0.17
120	2.00 ± 0.22	2.21 ± 0.00
135	2.08 ± 0.26	2.38 ± 0.06

TABLE 39 RETENTION OF ^{14}C-BHT IN COTTONSEED OIL AT 204 C

Time (hr)	Radioactive Antioxidant Retention (% of initial) ^{14}C-BHT[a]
0	100
0.5	30.5
1	11.9
2	6.7
3	5.2
6	4.8

[a]Initial count 2.1×10^3 cpm/0.1 ml cottonseed oil.

TABLE 40 CHEMICALLY MEASURED LEVELS OF BHA IN FISH STICKS DURING STORAGE AT -26 C AFTER INTENDED ADDITION OF 150 PPM ON A FAT BASIS

Storage Time (mo.)	Amount of BHA Measured (ppm on fat basis)
Initial	100 ± 20
3	96 ± 10
6	97 ± 17

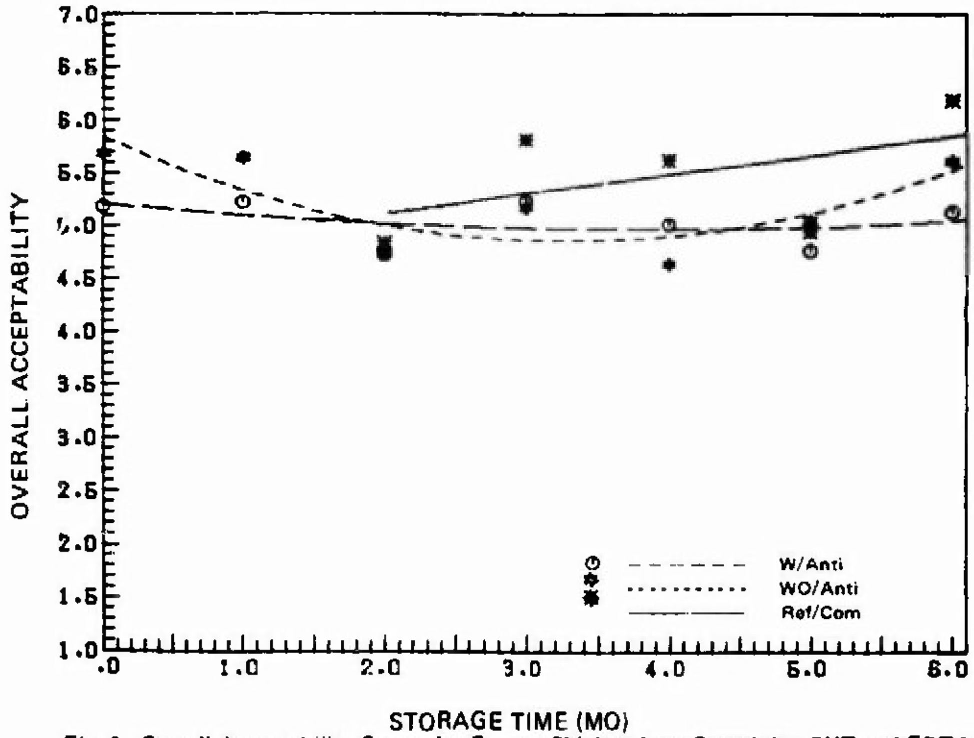


Fig. 2 Overall Acceptability Scores for Frozen Chicken Legs Containing BHT and EDTA During Storage for 6 Mo at -26 C.

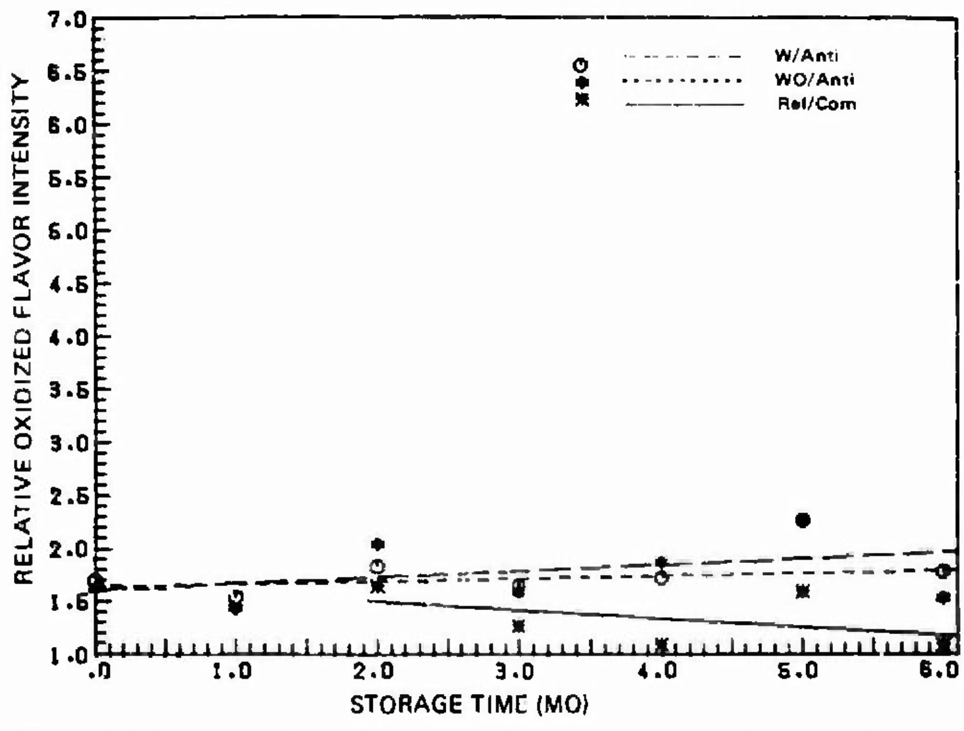


Fig. 3 Development of Oxidized Flavors for Frozen Chicken Legs Containing BHT and EDTA During Storage for 6 Mo at -26 C.

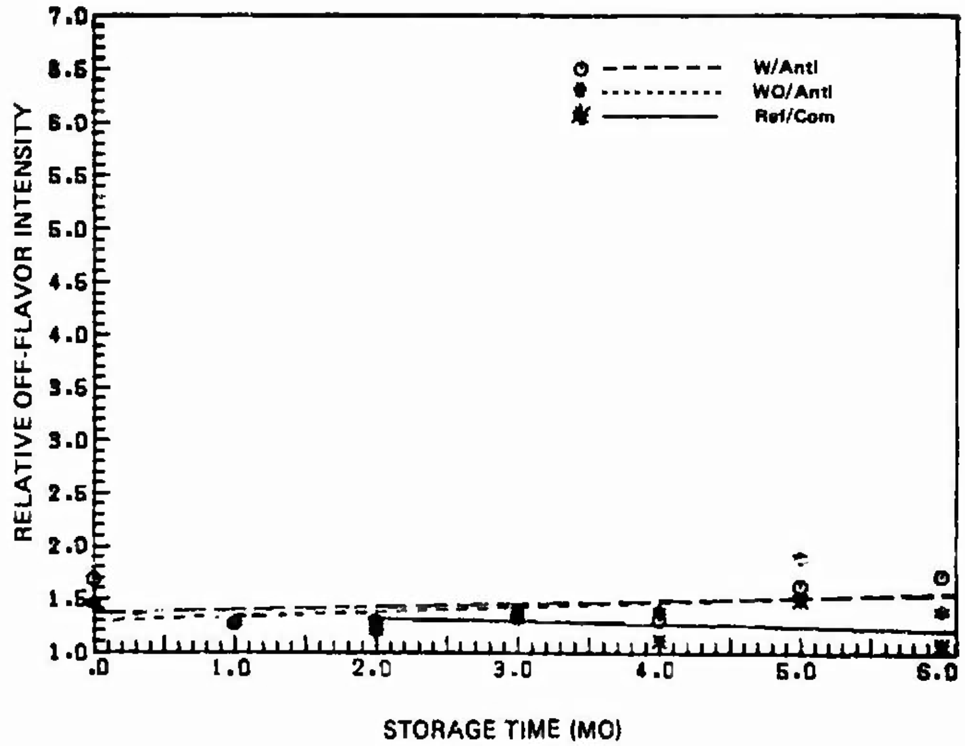


Fig. 4 Development of Off-Flavors for Frozen Chicken Legs Containing BHT and EDTA During Storage for 6 Mo at -26 C.

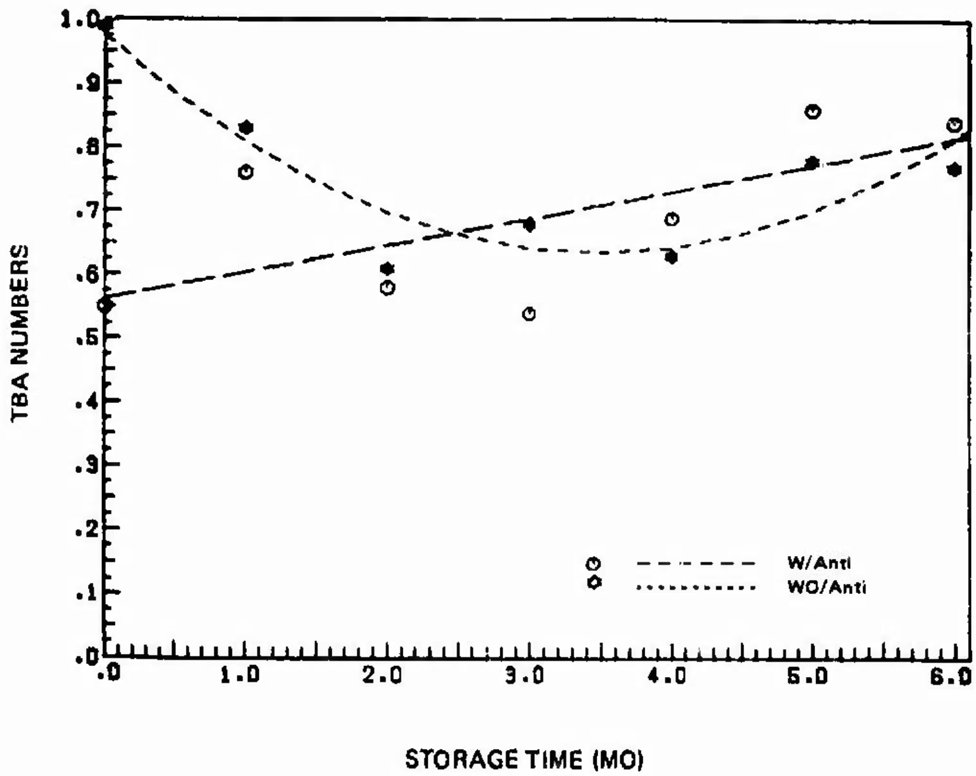


Fig. 5 TBA Values for Fr^zen Chicken Legs Containing BHT and EDTA During Storage for 6 Mo at -26 C.

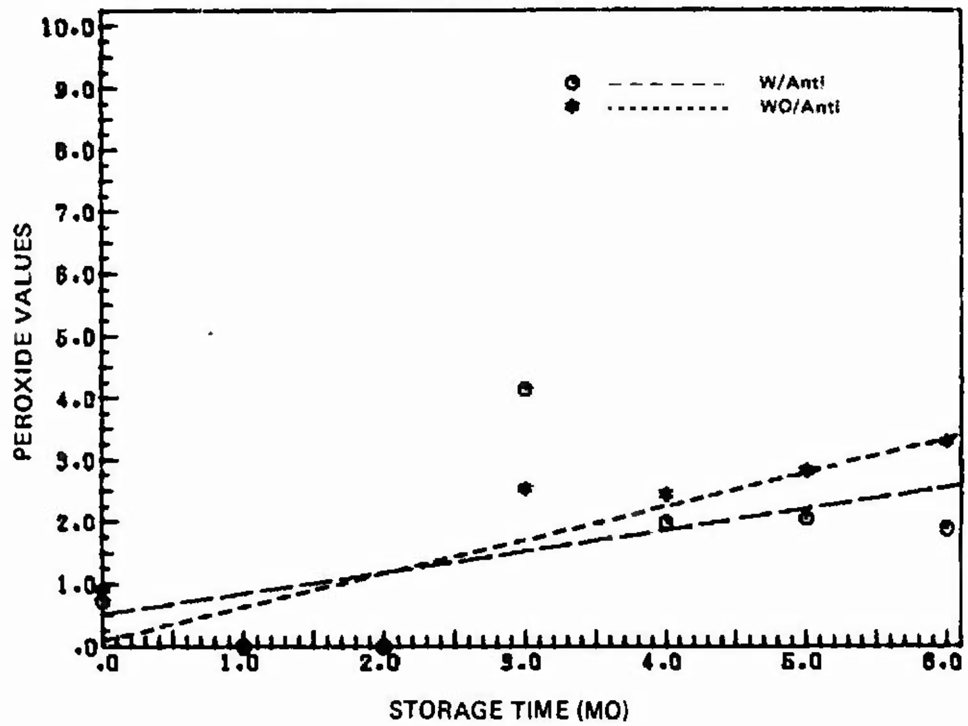


Fig. 6 Peroxide Values for Frozen Chicken Legs Containing BHT and EDTA During Storage for 6 Mo at -26 C.

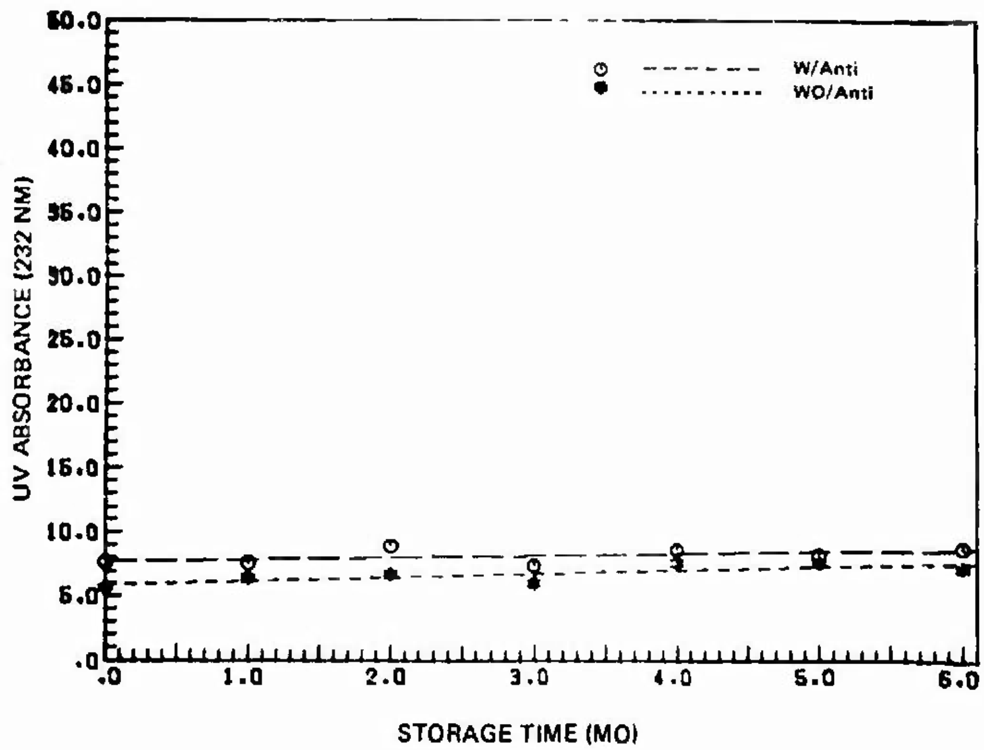


Fig. 7 UV Absorbance (232 nm) for Lipids from Frozen Chicken Legs Containing BHT and EDTA During Storage for 6 Mo at -26 C.

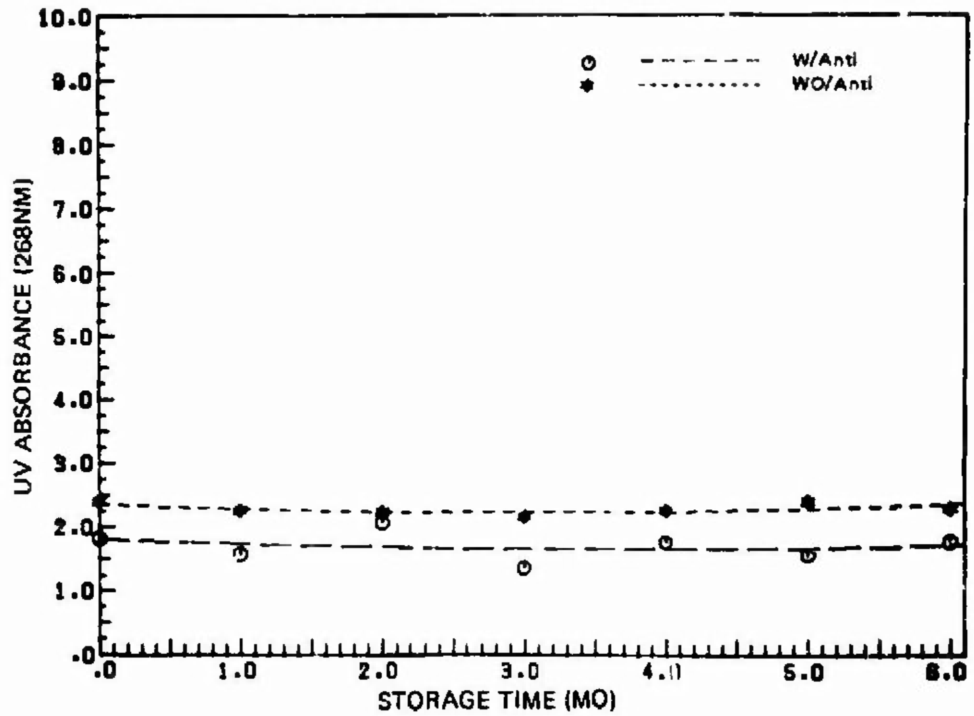


Fig. 8 UV Absorbance (268 nm) for Lipids from Frozen Chicken Legs Containing BHT and EDTA During Storage for 6 Mo at -26 C.

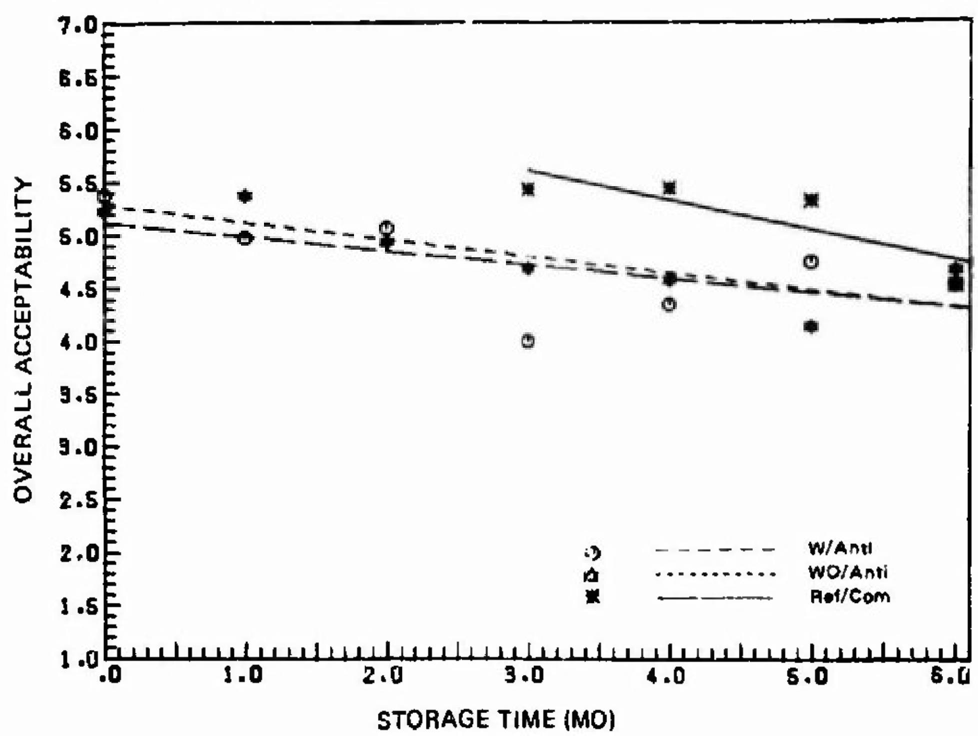


Fig. 9 Overall Acceptability Scores for Frozen Fish Sticks Containing BHA and Citric Acid During Storage for 6 Mo at -26 C.

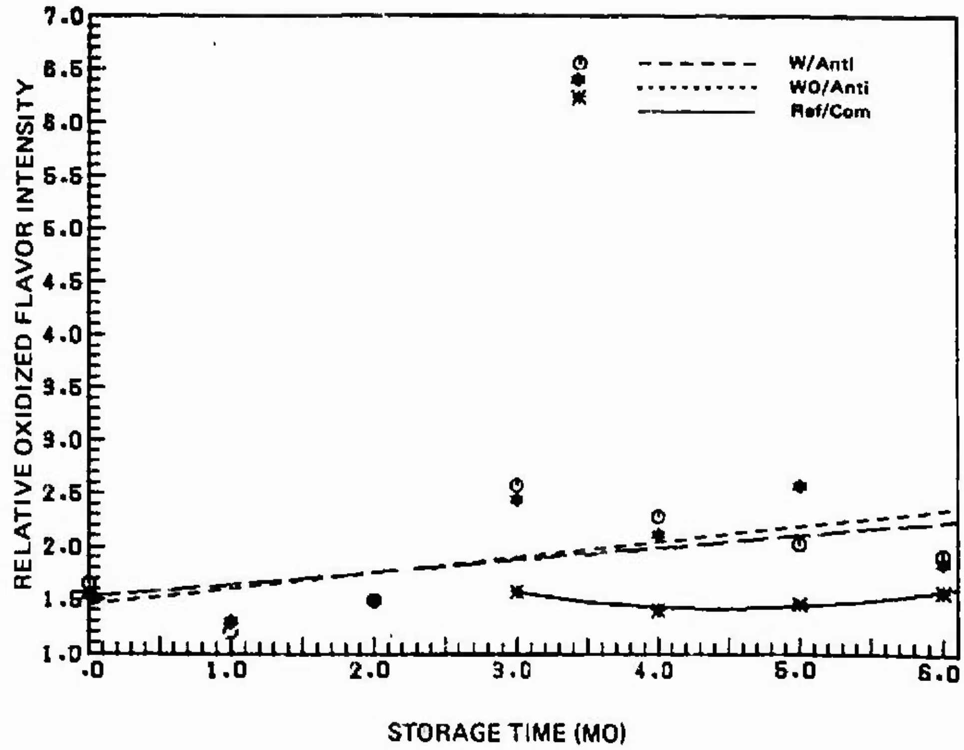


Fig. 10 Development of Oxidized Flavors for Frozen Fish Sticks Containing BHA and Citric Acid During Storage for 6 Mo at -26 C.

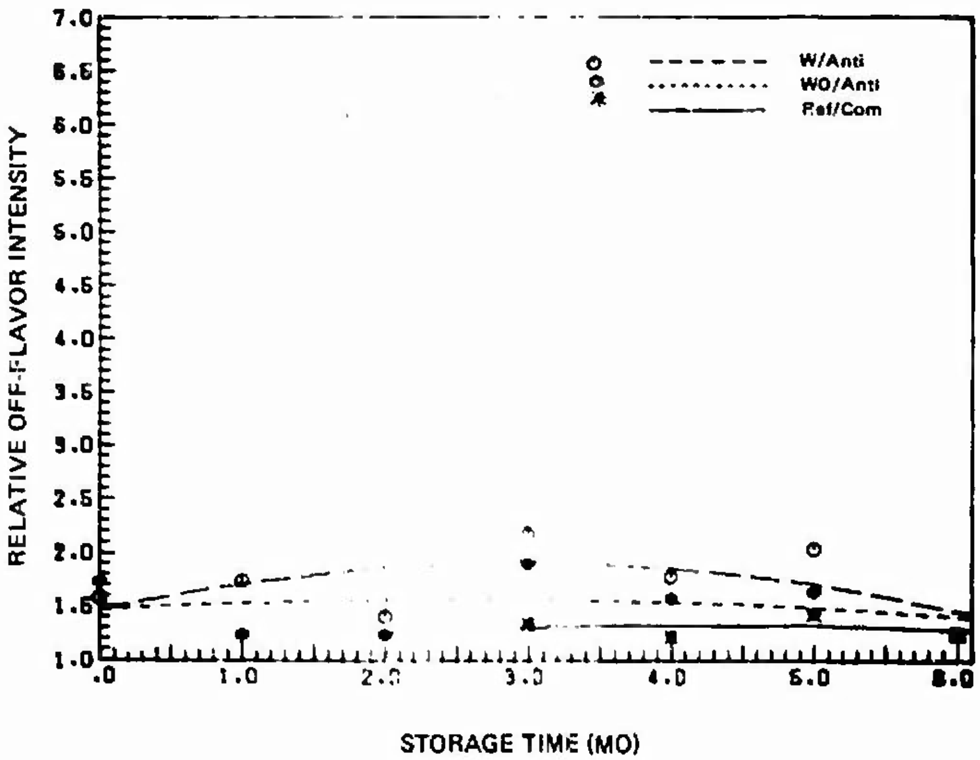
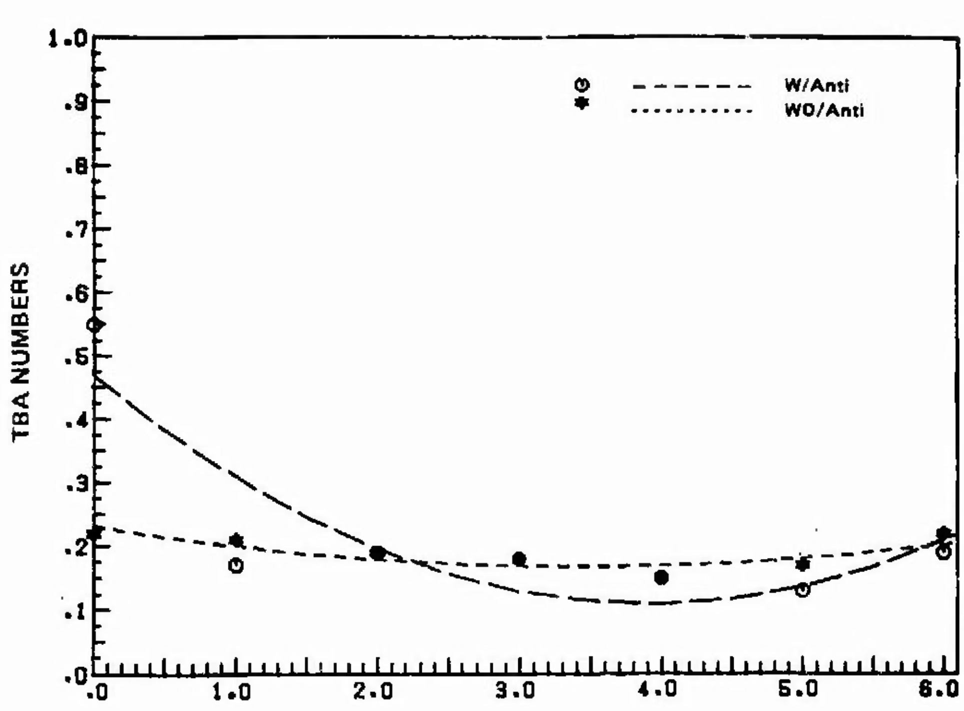
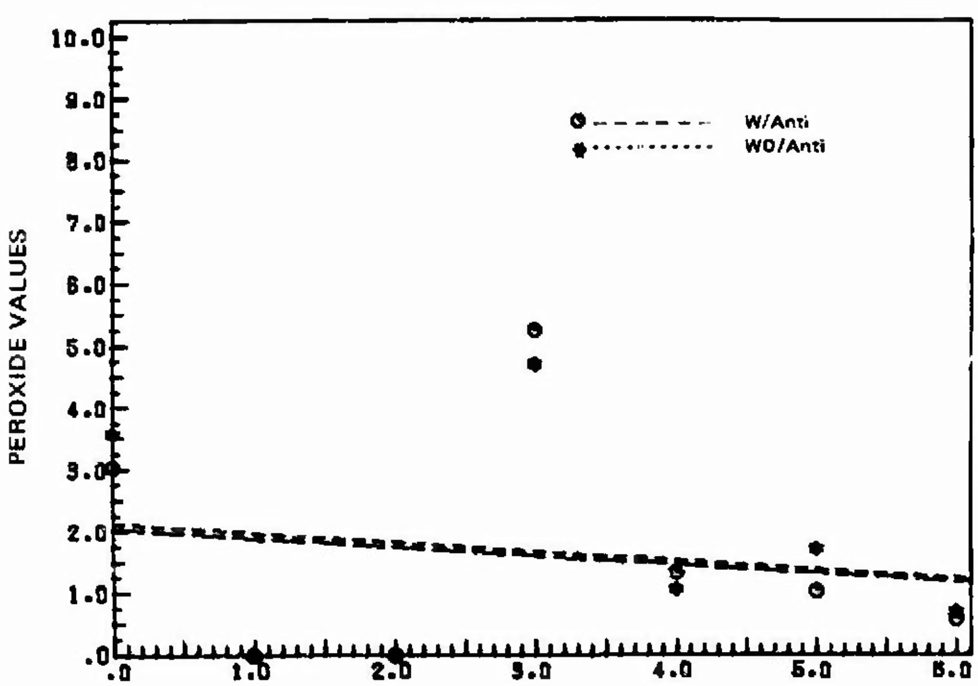


Fig. 11 Development of Off-Flavors in Frozen Fish Sticks Containing BHA and Citric Acid During 6 Mo Storage at -26 C.



STORAGE TIME (MO)

Fig. 12 TBA Values for Frozen Fish Sticks Containing BHA and Citric Acid During Storage for 6 Mo at -26 C.



STORAGE TIME (MO)

Fig. 13 Peroxide Values for Frozen Fish Sticks Containing BHA and Citric Acid During 6 Mo Storage at -26 C.

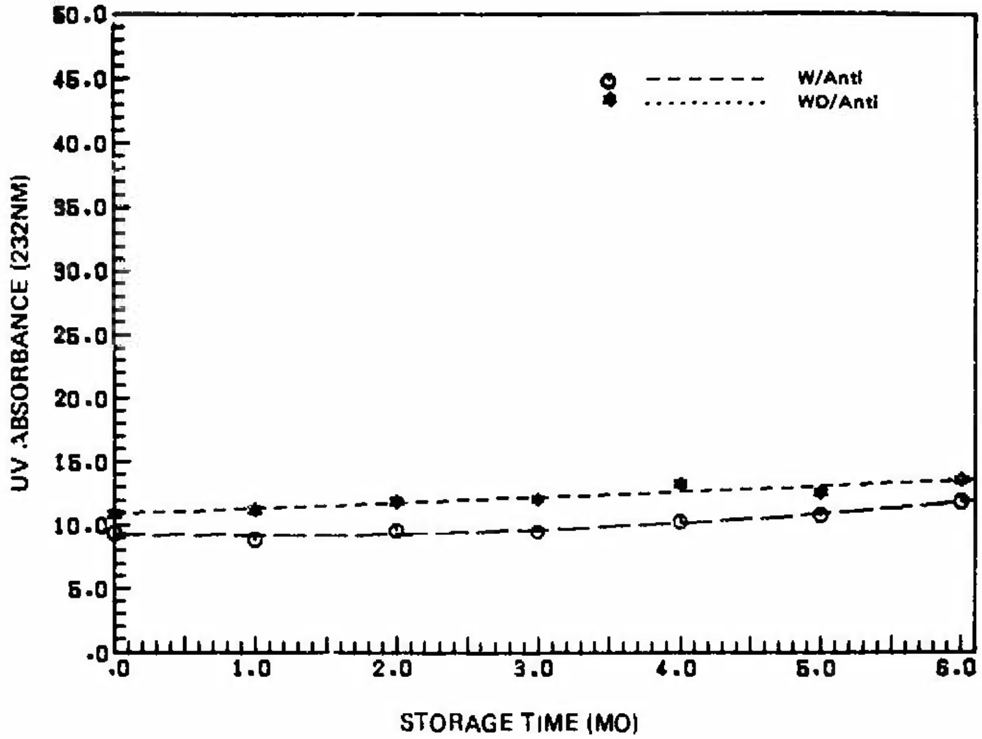


Fig. 14 UV Absorbance (232 nm) for Lipids from Frozen Fish Sticks Containing BHA and Citric Acid During 6 Mo Storage at -26 C.

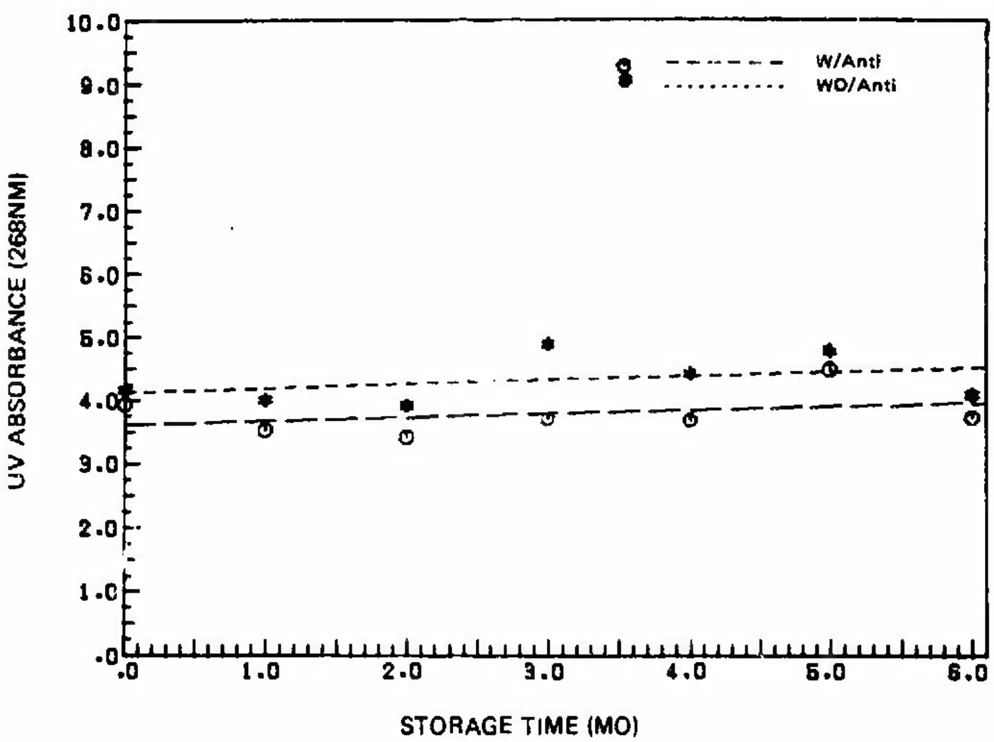


Fig. 15 UV Absorbance (268 nm) for Lipids from Frozen Fish Sticks Containing BHA and Citric Acid During 6 Mo Storage at -26 C.

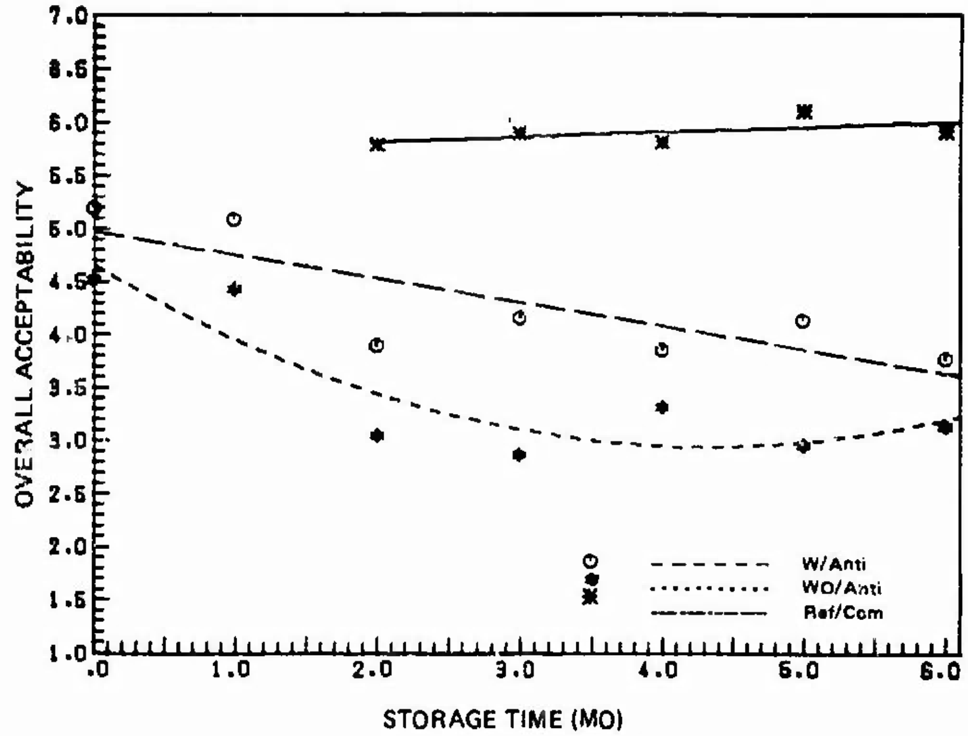


Fig. 16 Overall Acceptability Scores for Frozen Frankfurters Containing BHT and EDTA During Storage for 6 Mo at -26 C.

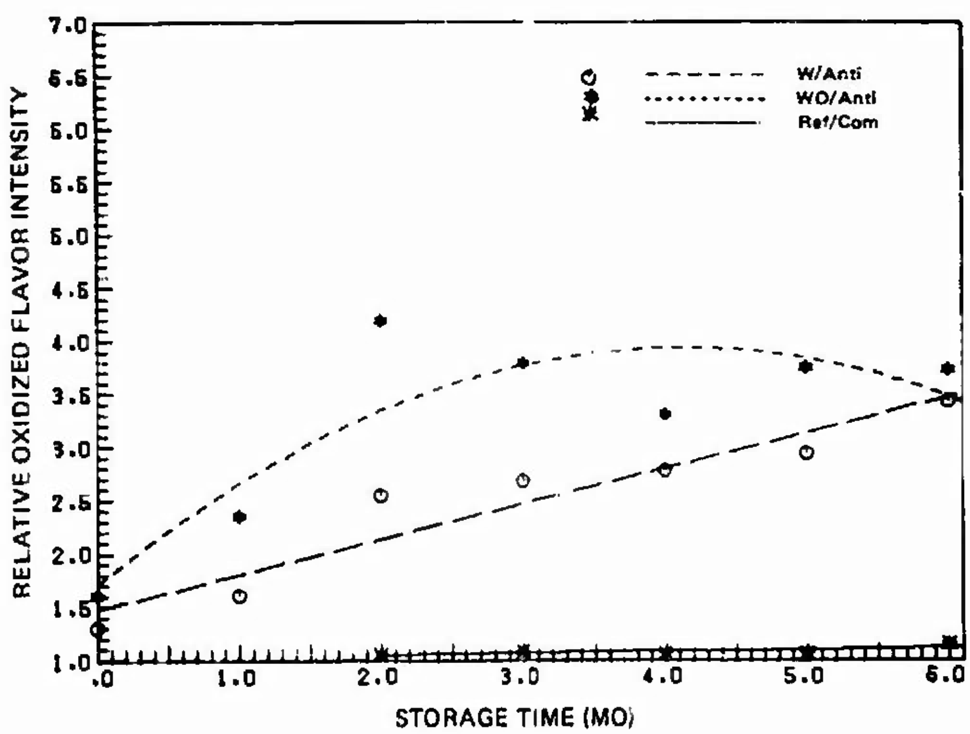


Fig. 17 Development of Oxidized Flavors for Frozen Frankfurthers Containing BHT and EDTA During Storage for 6 Mo at -26 C.

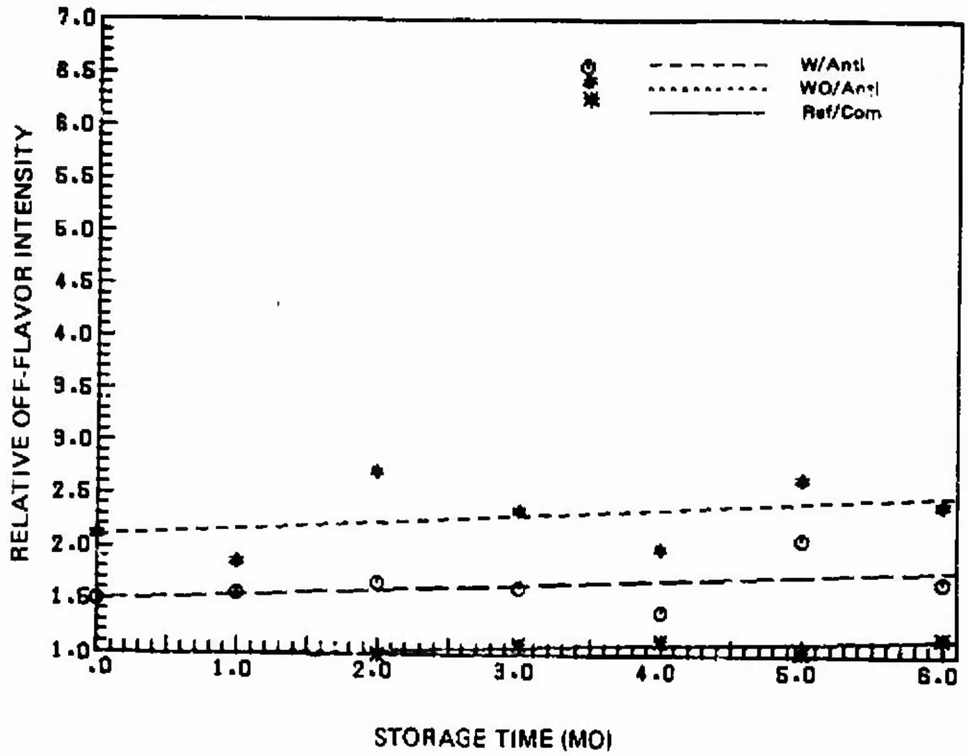


Fig. 18 Development of Off-Flavors for Frozen Frankfurthers Containing BHT and EDTA During Storage for 6 Mo at -26 C.

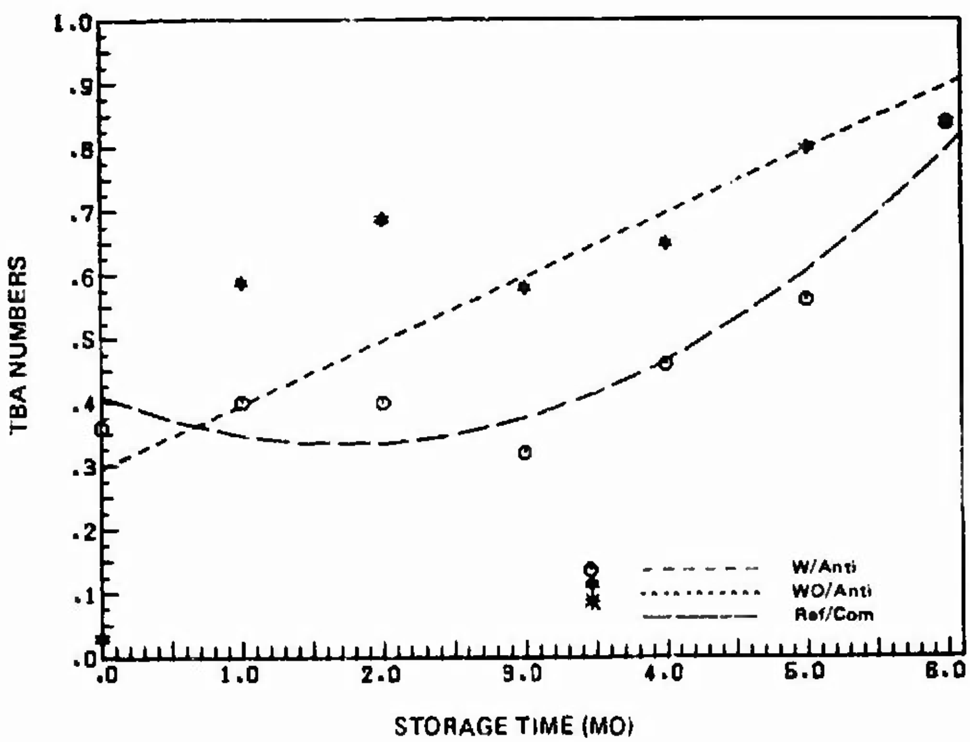


Fig. 19 TBA Values for Frozen Frankfurters Containing BHT and EDTA During Storage for 6 Mo at -26 C.

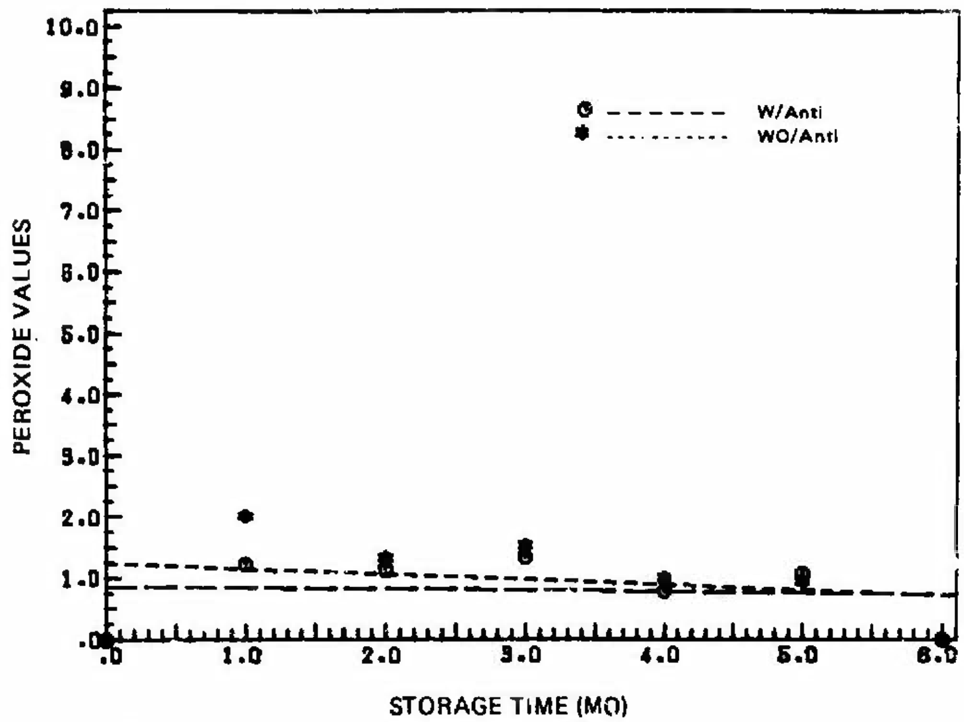


Fig. 20 Peroxide Values for Frozen Frankfurters Containing BHT and EDTA During Storage for 6 Mo at -26 C.

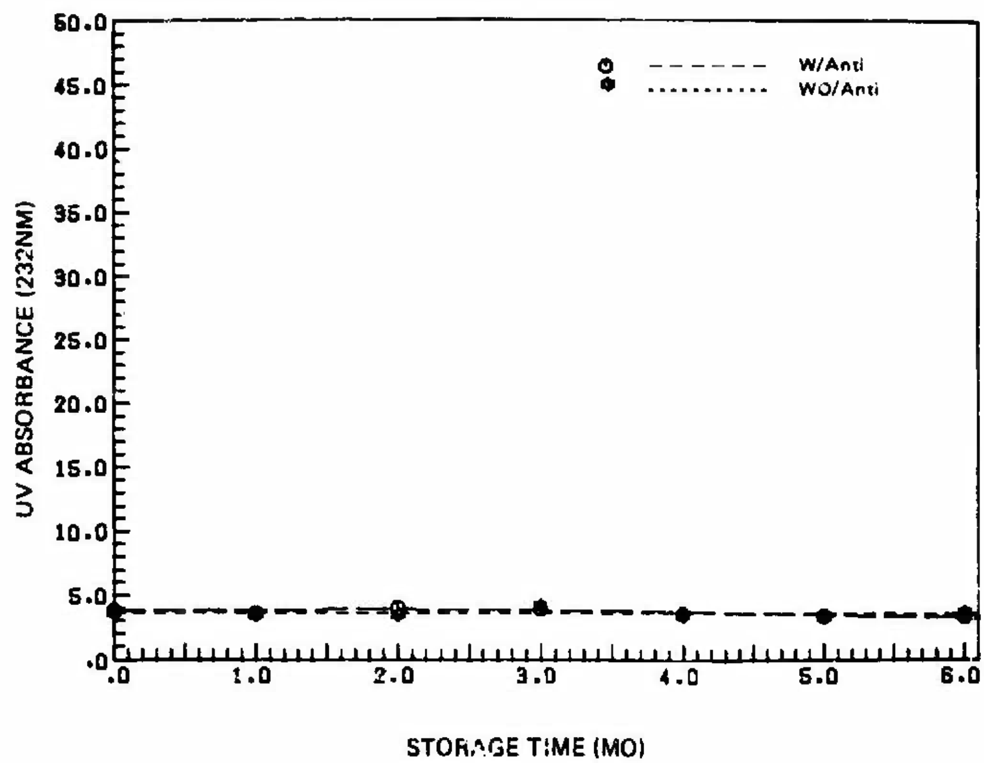


Fig. 21 UV Absorbance (232 nm) for Lipids from Frozen Frankfurters Containing BHT and EDTA During Storage for 6 Mo at -26 C.

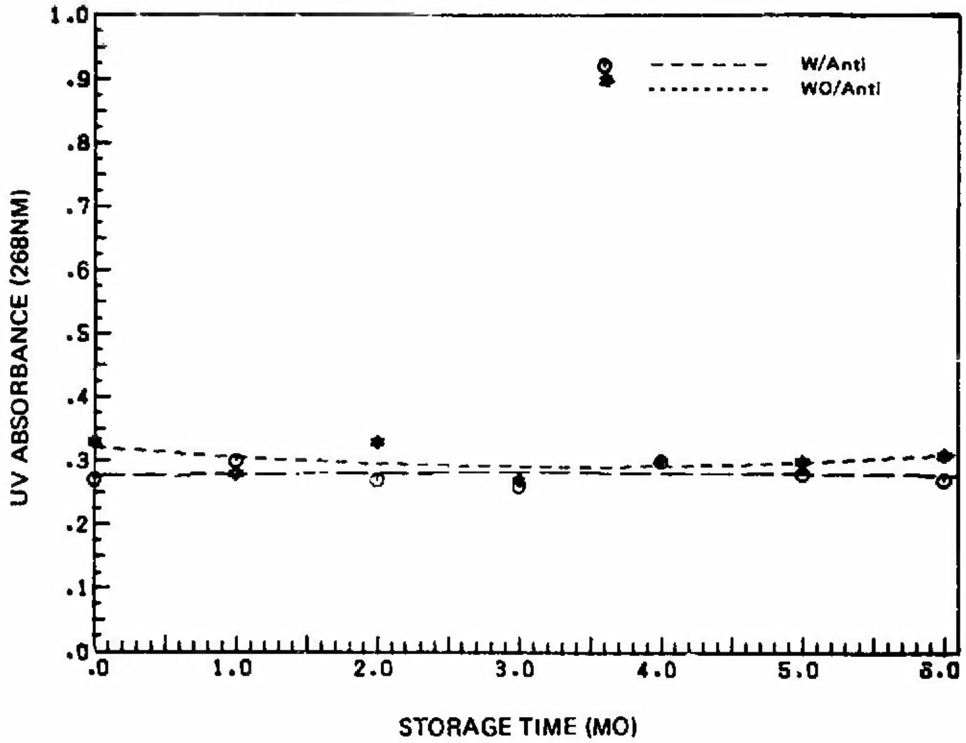


Fig. 22 UV Absorbance (268 nm) for Lipids from Frozen Frankfurters Containing BHT and EDTA During Storage for 6 Mo at -26 C.

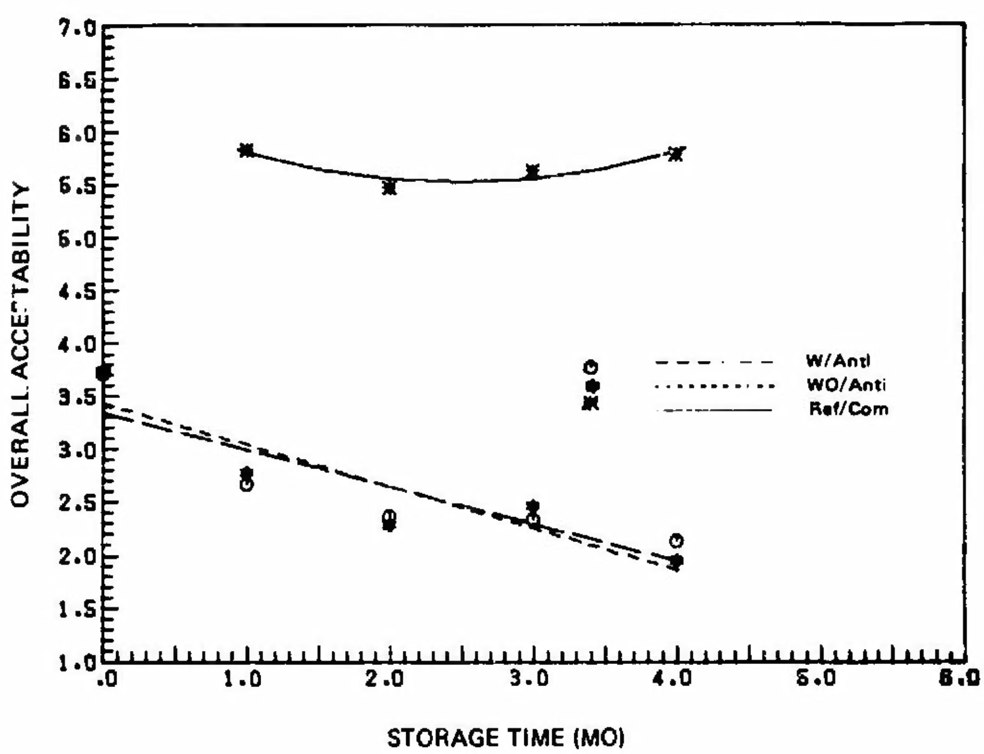


Fig. 23 Overall Acceptability Scores for Freeze Dried Pork Chops Containing BHA and Citric Acid During Storage for 4 Mo at 32 C.

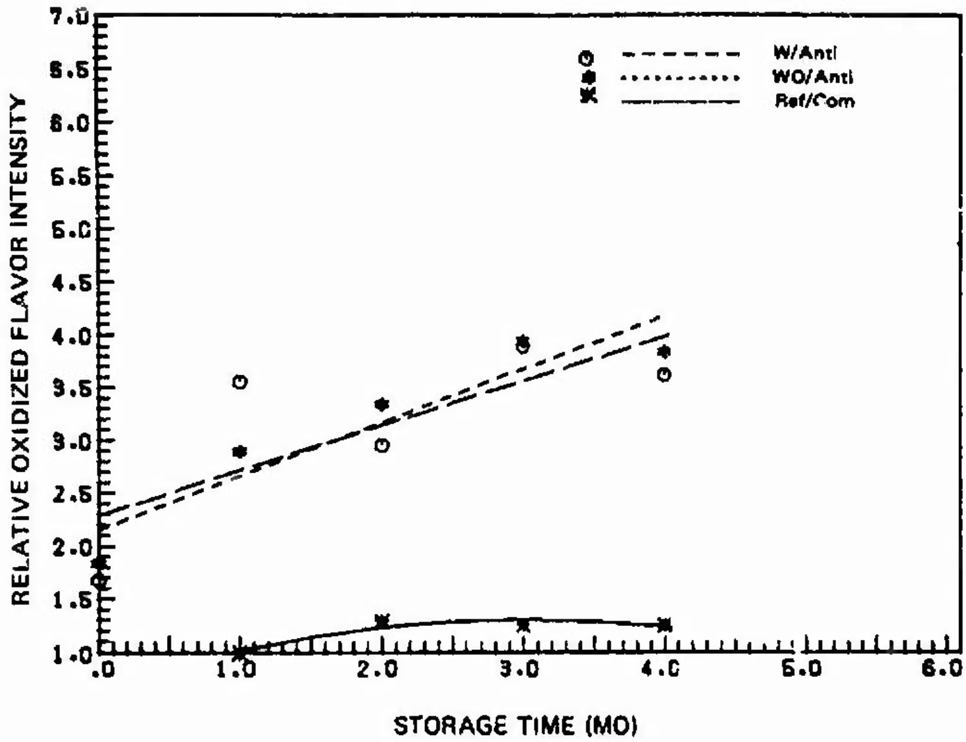


Fig. 24 Development of Oxidized Flavors for Freeze Dried Pork Chops Containing BHA and Citric Acid During Storage for 4 Mo at 32 C.

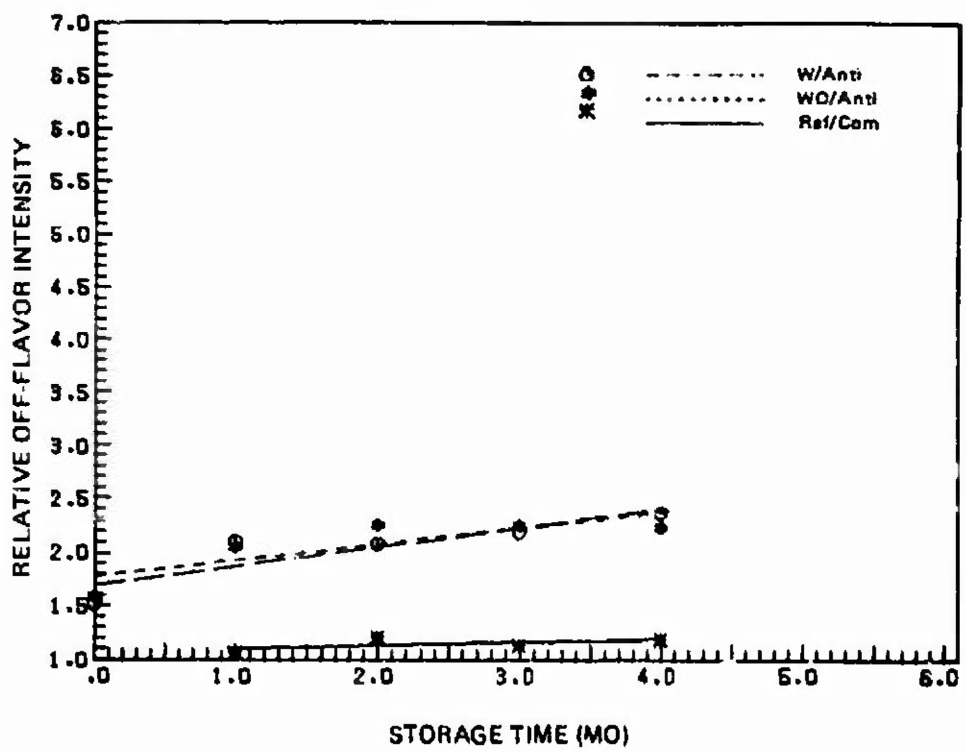


Fig. 25 Development of Off-Flavors for Freeze Dried Pork Chops Containing BHA and Citric Acid During Storage for 4 Mo at 32 C.

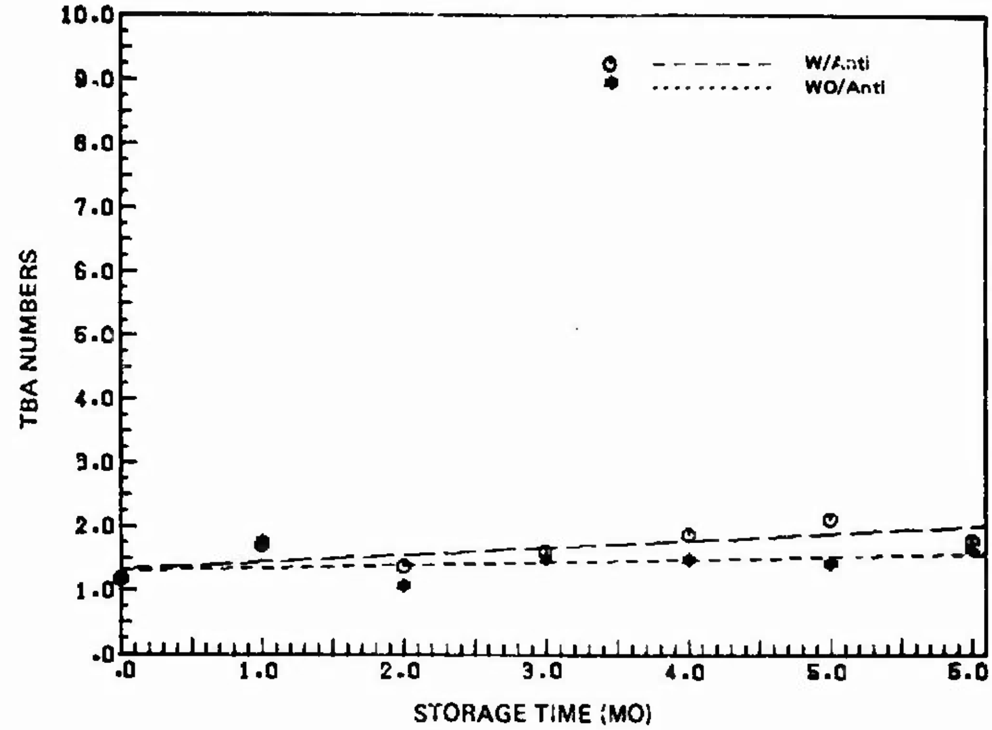


Fig. 26 TBA Values for Freeze Dried Pork Chops Containing BHA and Citric Acid During Storage for 6 Mo at 32 C.

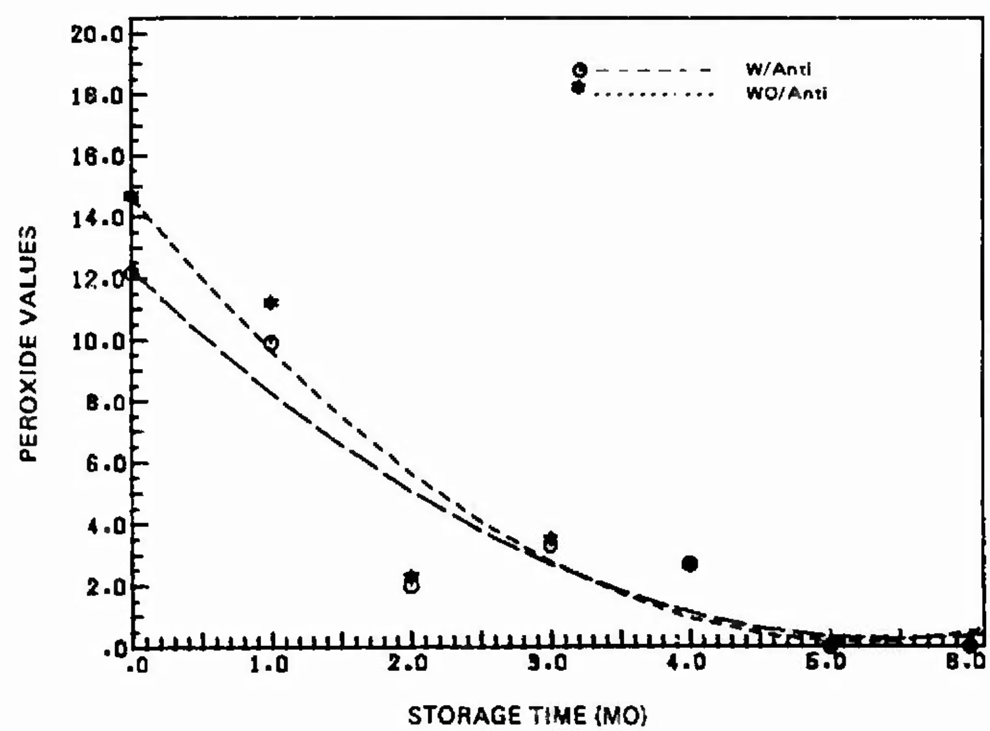


Fig. 27 Peroxide Values for Freeze Dried Pork Chops Containing BHA and Citric Acid During Storage for 6 Mo at 32 C.

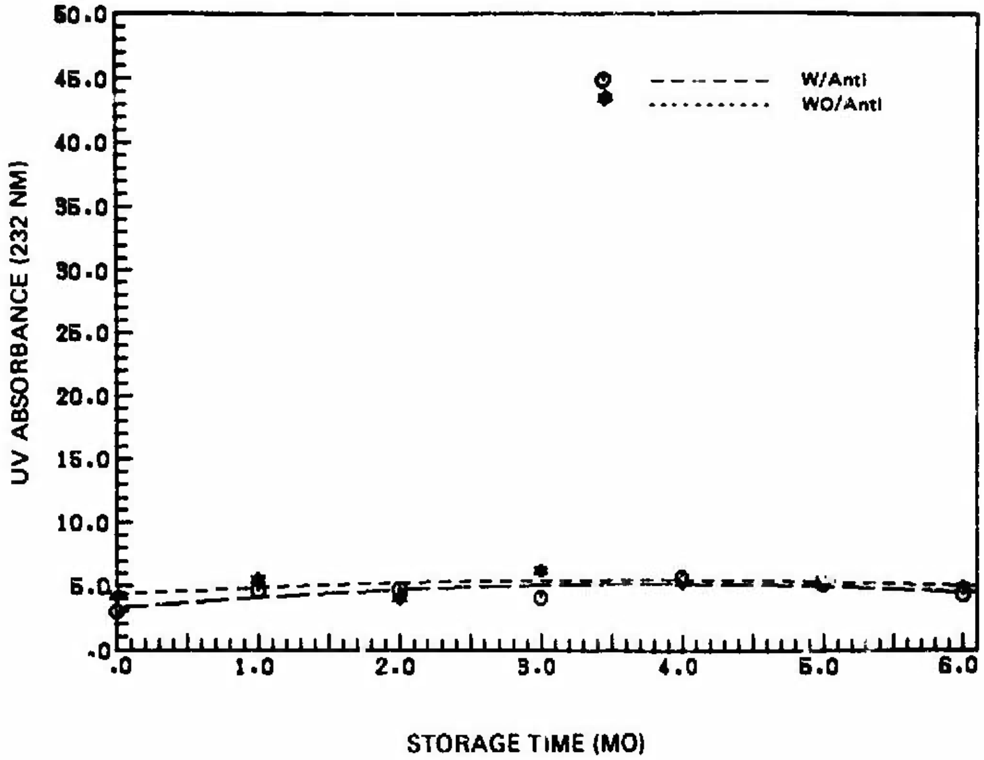


Fig. 28 UV Absorbance (232 nm) for Lipids from Freeze Dried Pork Chops Containing BHA and Citric Acid During Storage for 6 Mo at 32 C.

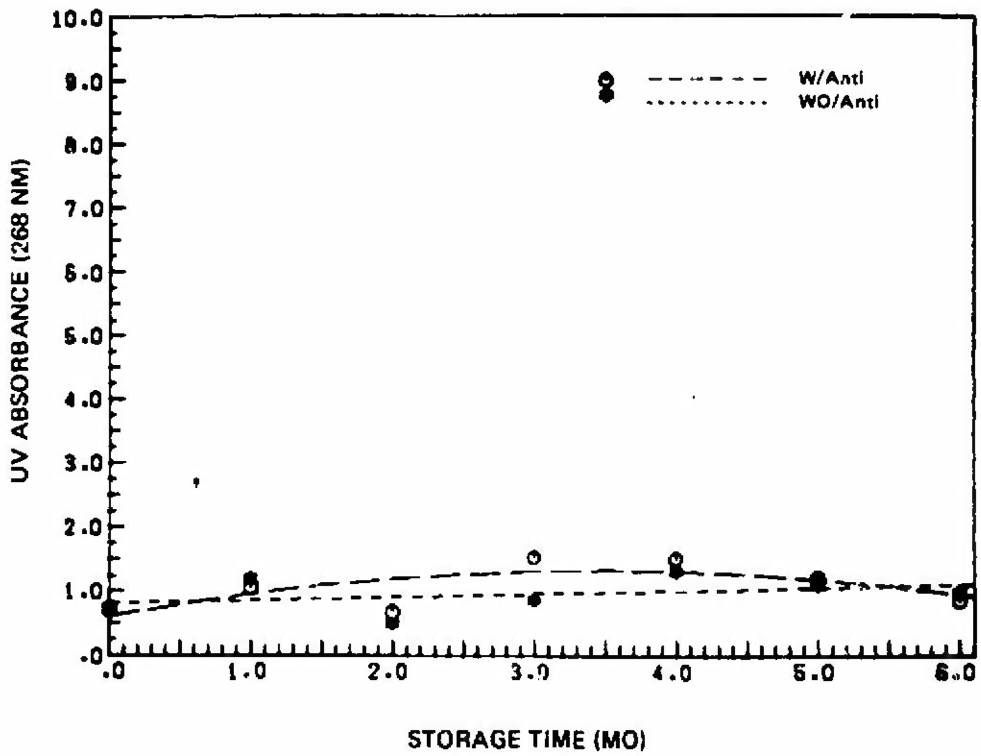


Fig. 29 UV Absorbance (268 nm) for Lipids from Freeze Dried Pork Chops Containing BHA and Citric Acid During Storage for 6 Mo at 32 C.

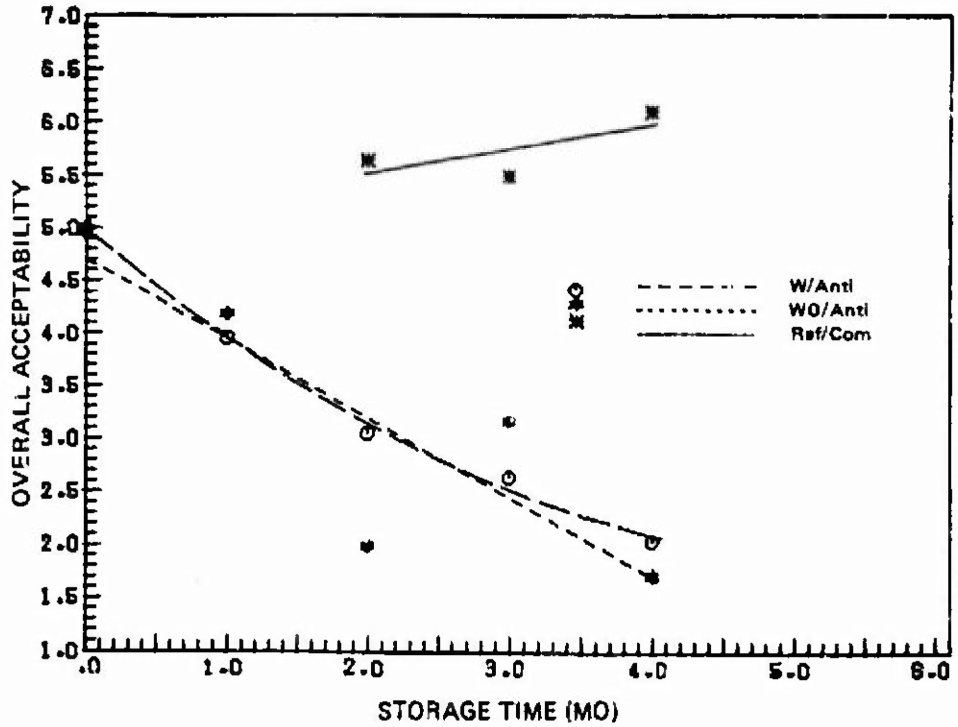


Fig. 30 Overall Acceptability Scores for Freeze Dried Beef Stew Containing BHA and Citric Acid During Storage for 4 Mo at 32 C.

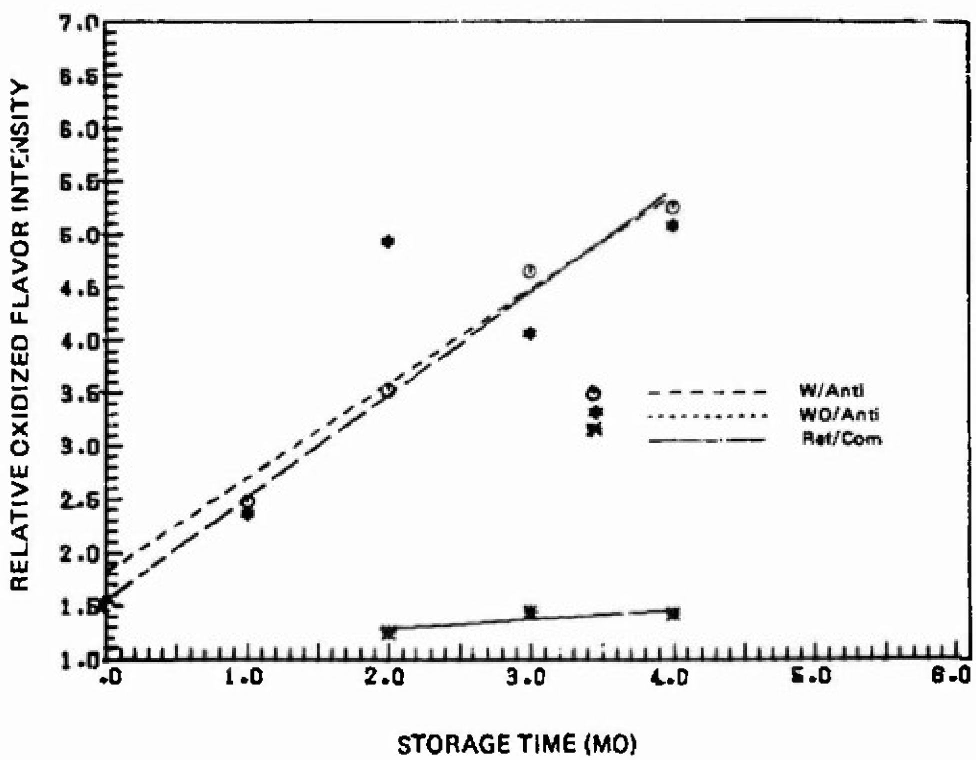


Fig. 31 Development of Oxidized Flavors for Freeze Dried Beef Stew Containing BHA and Citric Acid During Storage for 4 Mo at 32 C.

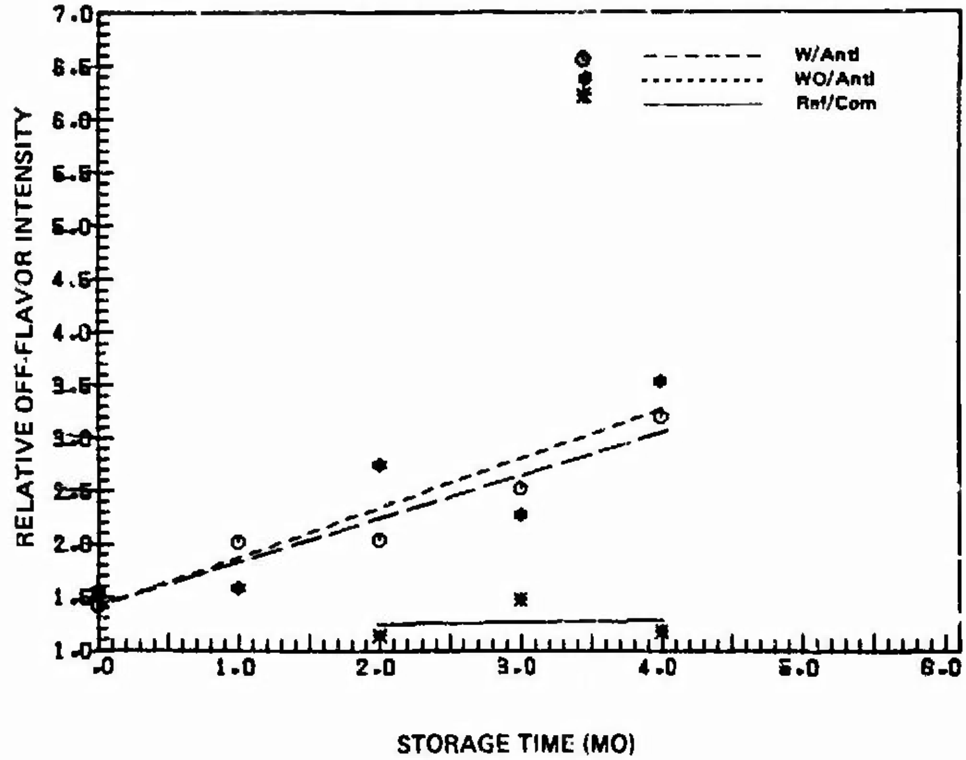


Fig. 32 Development of Off-Flavors for Freeze Dried Beef Stew Containing BHA and Citric Acid During Storage for 4 Mo at 32 C.

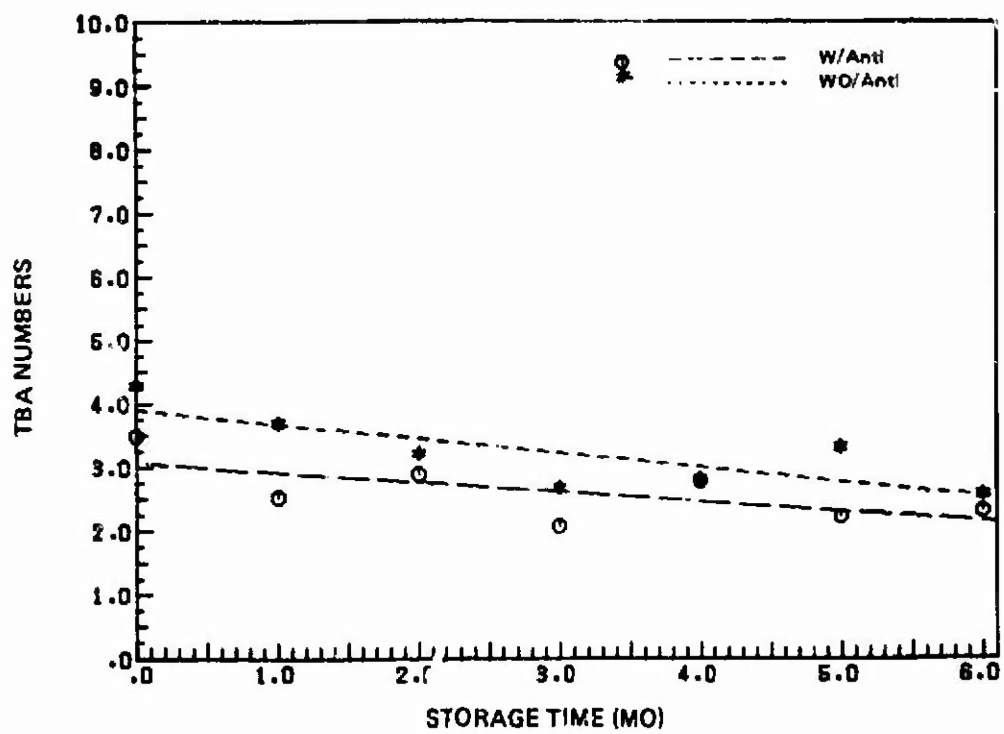


Fig. 33 TBA Values for Freeze Dried Beef Stew Containing BHA and Citric Acid During Storage for 6 Mo at 32 C.

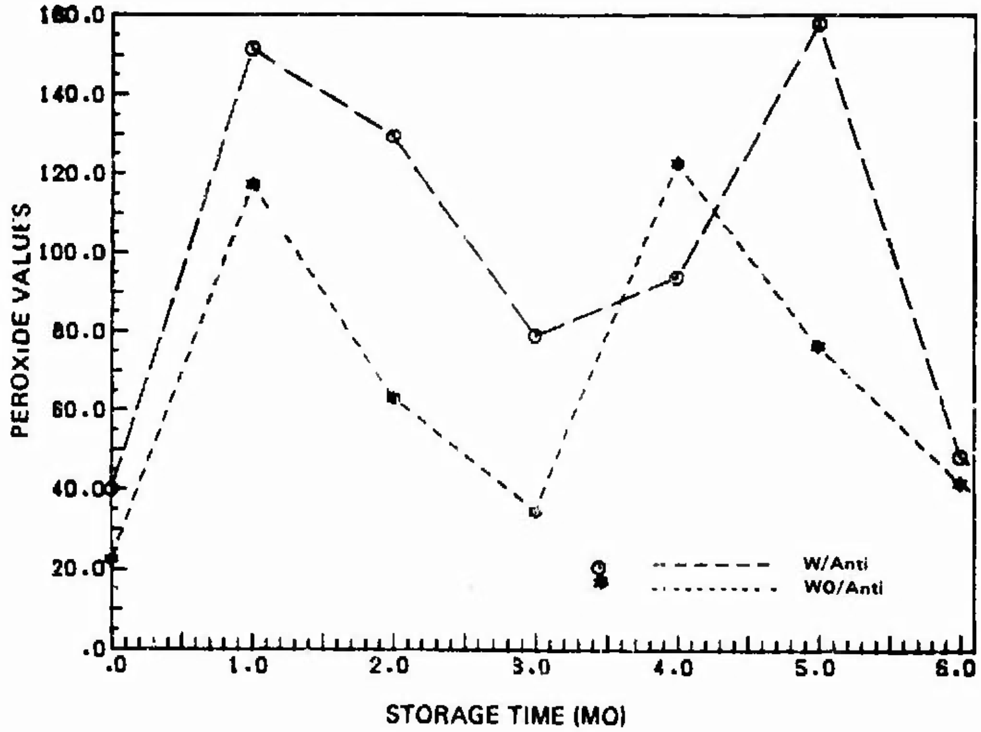


Fig. 34 Peroxide Values for Freeze Dried Beef Stew Containing BHA and Citric Acid During Storage for 6 Mo at 32 C.

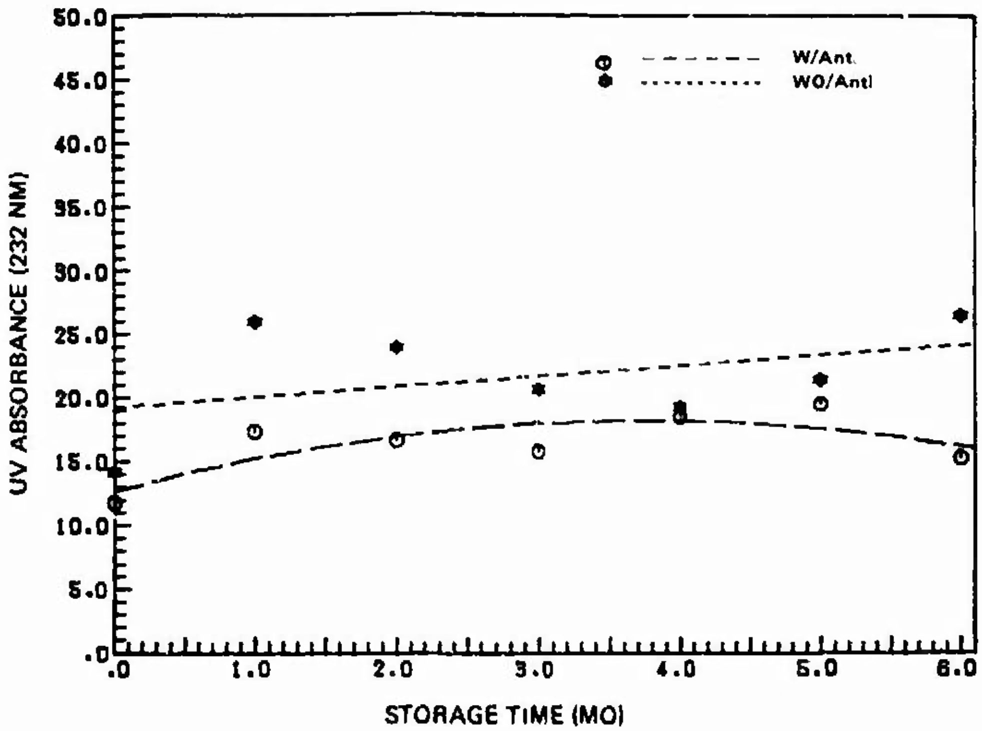


Fig. 35 UV Absor.ance (232 nm) for Lipids from Freeze Dried Beef Stew Containing BHA and Citric Acid During Storage for 6 Mo at 32 C.

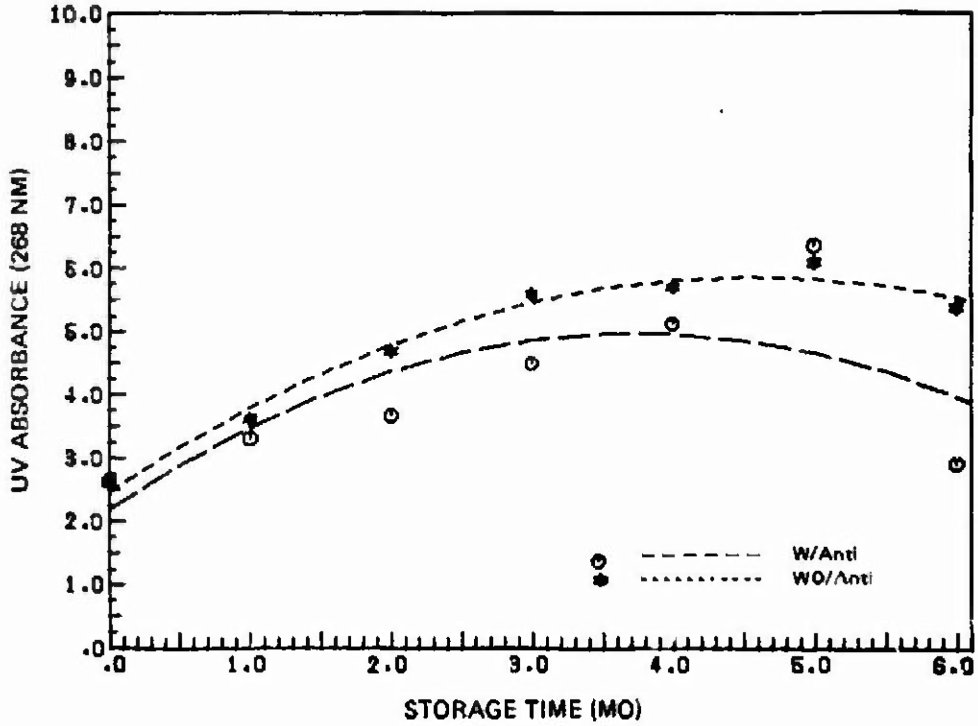


Fig. 36 UV Absorbance (268 nm) for Lipids from Freeze Dried Beef Stew Containing BHA and Citric Acid During Storage for 6 Mo at 32 C.

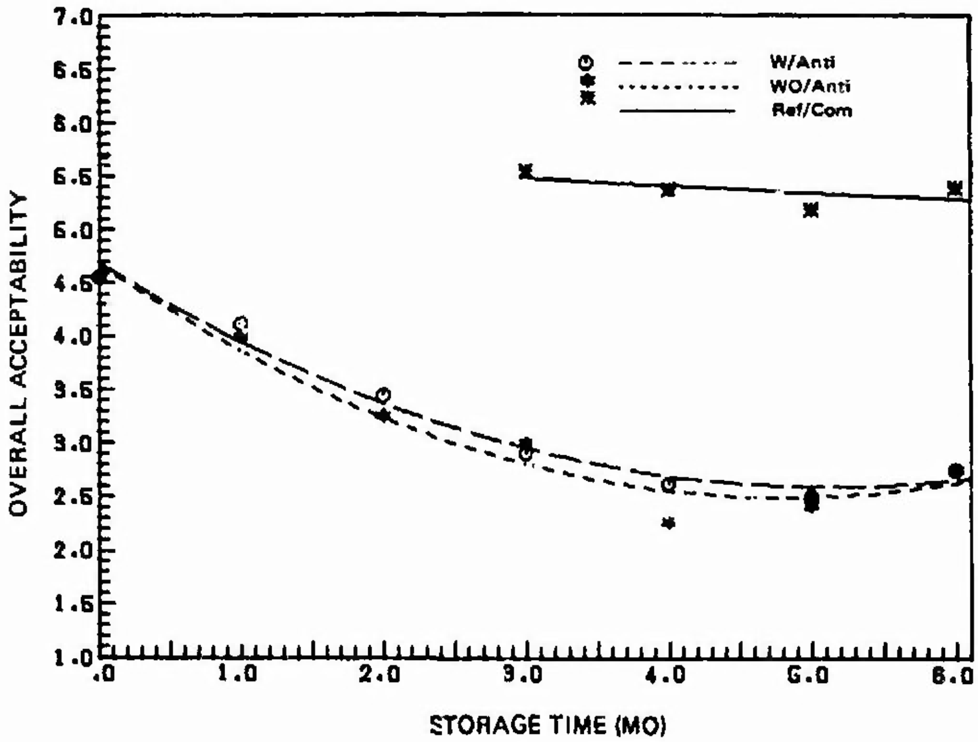
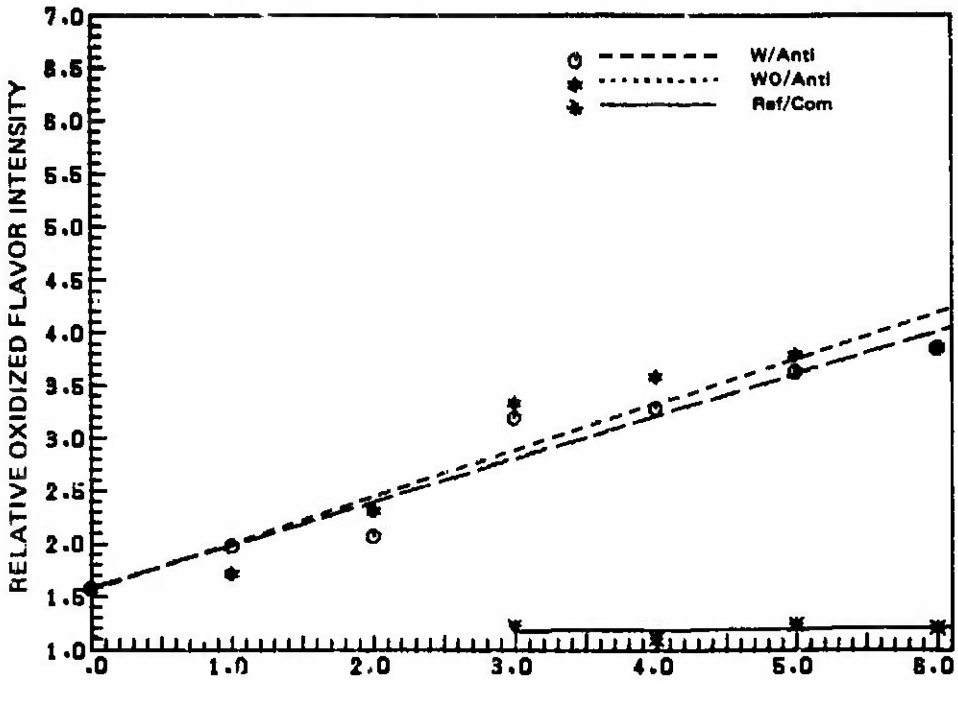
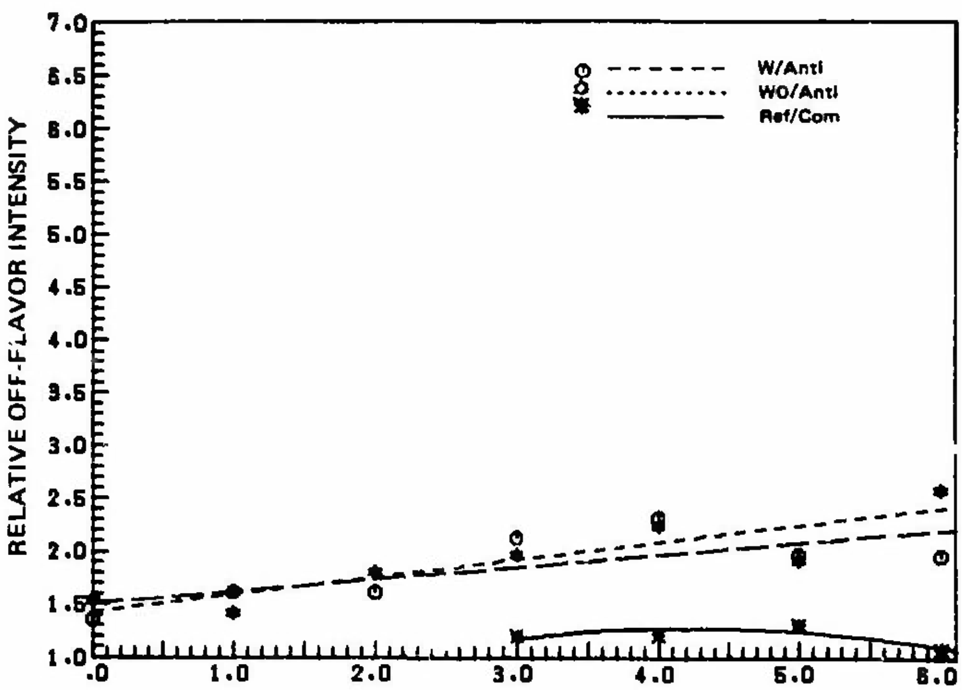


Fig. 37 Overall Acceptability Scores for Freeze Dried Carrots Containing PG and Citric Acid During Storage for 6 Mo at 32 C.



STORAGE TIME (MO)

Fig. 38 Development of Oxidized Flavors for Freeze Dried Carrots Containing PG and Citric Acid During Storage for 6 Mo at 32 C.



STORAGE TIME (MO)

Fig. 39 Development of Off-Flavors for Freeze Dried Carrots Containing PG and Citric Acid During Storage for 6 Mo at 32 C.

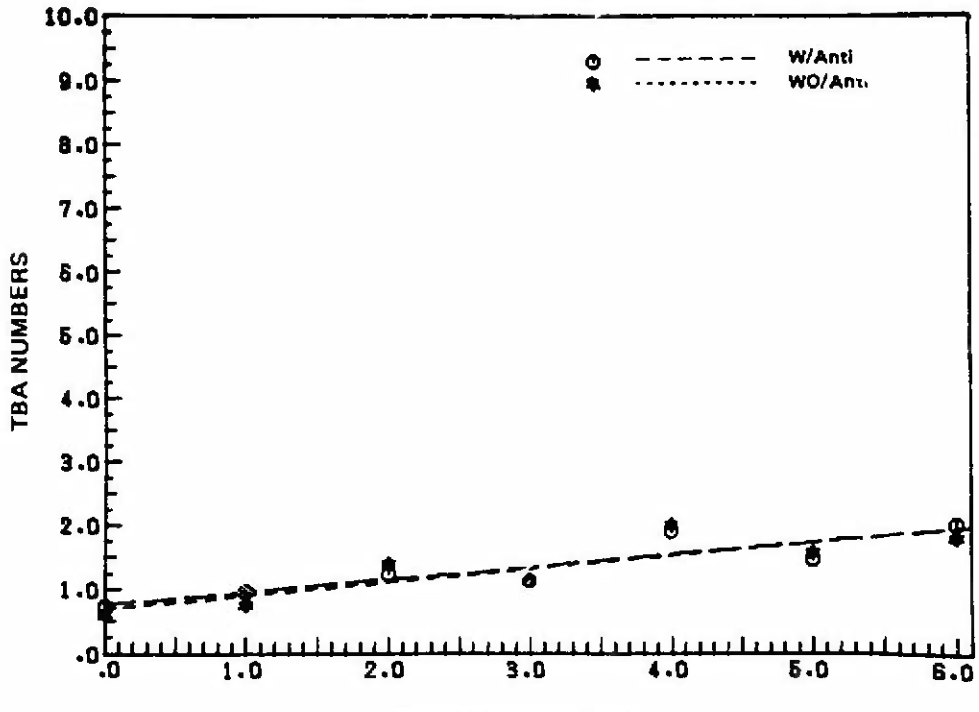


Fig. 40 TBA Values for Freeze Dried Carrots Containing PG and Citric Acid During Storage for 6 Mo at 32 C.

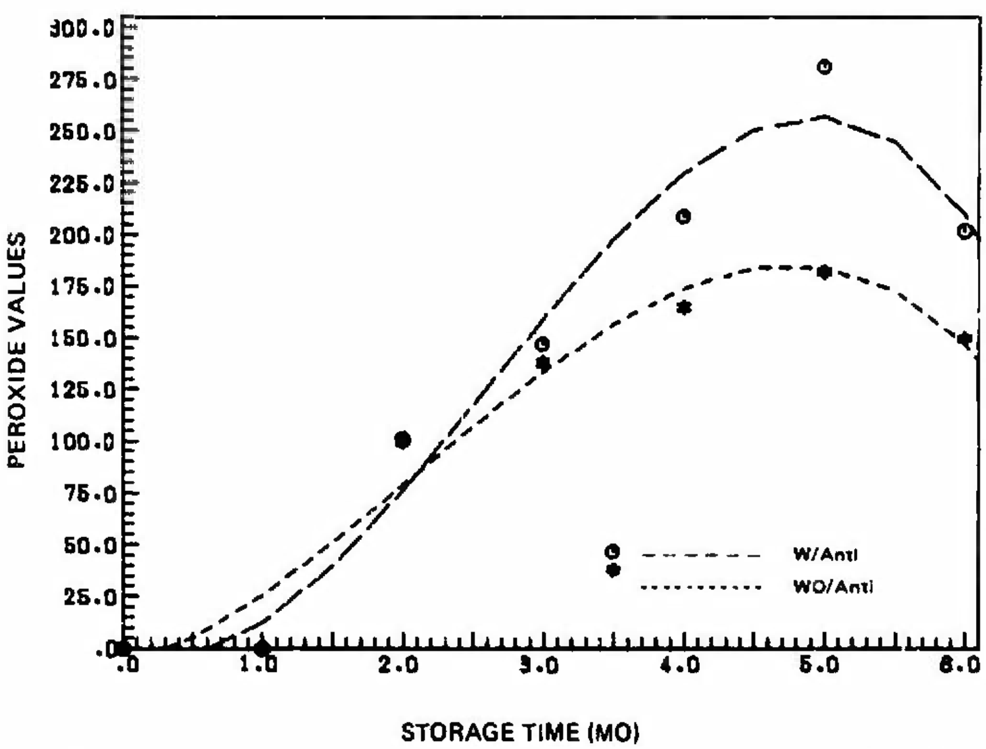


Fig. 41 Peroxide Values for Freeza Dried Carrots Containing PG and Citric Acid During Storage for 6 Mo at 32 C.

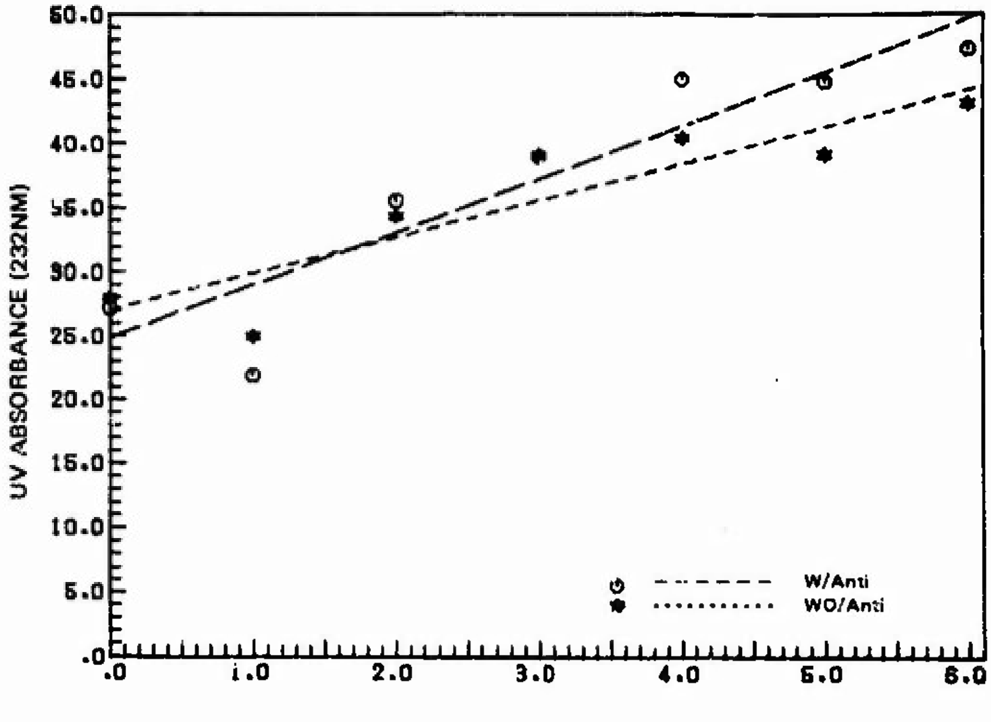


Fig. 42 UV Absorbance (232 nm) for Lipids from Freeze Oried Carrots Containing PG and Citric Acid During Storage for 6 MO at 32 C.

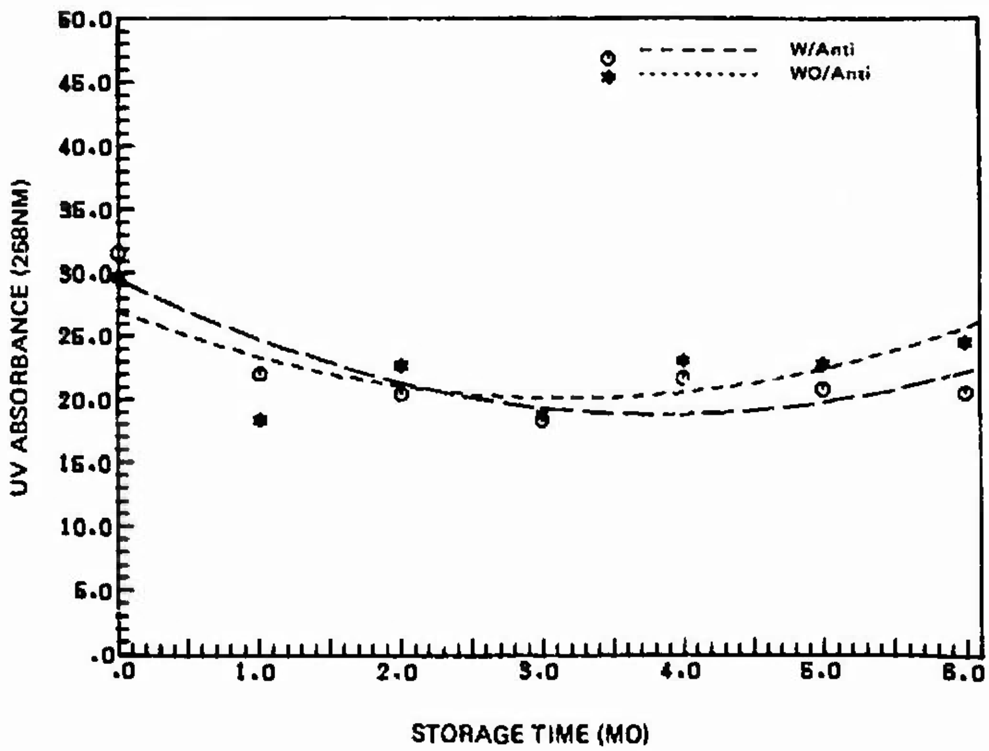


Fig. 43 UV Absorbance (268 nm) for Lipids from Free?e Oried Carrots Containing PG and Citric Acid During Storage for 6 Mo at 32 C.

IV. Discussion

A. Introduction of Antioxidants into Chicken Legs.

Breaded, precooked chicken legs were chosen as a model for multi-processed meat products exhibiting high A_w, relatively high fat levels (chicken only, 10.6%, USDA Handbook No. 8 (7)), heterogeneous fat distribution; this study, (breading-19.7%, skin-22.6%, and muscle-3.5%), and tendencies to develop distinct undesirable oxidized-lipid and warmed-over flavors. The antioxidant delivery systems investigated (Table 1) reflect those compatible with current processing practices as well as newer more innovative procedures.

The data obtained for the retention of radioactive antioxidants through processing based on the amounts applied initially and that remaining in finished breaded chicken legs are presented in Table 3. Further post-application processing was not a factor in retention of antioxidants when they were applied by spraying before freezing, in the frying oil, or in immersion freezing because there was no further opportunity for incurring losses. In comparing retentions of antioxidants when applied by spraying before battering to batter incorporation, ^{14}C-BHT exhibited the greatest overall retention and was essentially all retained. On the other hand, the other phenolic antioxidants (^{3}H-PG and ^{3}H-BHA) were poorly retained, but applying with the batter appeared to result in slightly greatly retention. Some of the losses of ^{3}H-BHA and ^{3}H-PG could have been due to oil extraction during frying. However, in chicken legs, ^{14}C-BHT which should be more oil-soluble (non-polar) did not decrease to an appreciable extent.

Therefore, direct volatilization and steam distillation of ^{3}H-BHA (M.P.59-60 C) during frying appear to be more attractive explanations and would be in agreement with mechanisms of loss discussed by Stuckey (18).

It should be pointed out that for this study it was assumed that measurable ^{3}H-radioactivity was directly attributable to labeled BHA and PG molecules. However, ^{3}H-exchange between ^{3}H-PG and ^{3}H-BHA and the environmental milieu under some conditions employed in this study, and especially at elevated temperatures, could possibly affect the data interpretations. While data collected for ^{3}H-labeled antioxidants should be cautiously interpreted, it does provide a means for following antioxidants in foods. The rates of ^{3}H-exchange for ^{3}H-PG and ^{3}H-BHA in aqueous systems at 5 C (Table 36) showed that ^{3}H-BHA was relatively stable in the pH range 5.0 to 7.0. On the other hand, ^{3}H-PG with three hydroxyl protons showed significant exchange rates, especially at the higher pH value (7.0).

For the chelating-type antioxidants, ^{14}C-CA and ^{14}C-EDTA application by spraying before battering results in considerably higher retention than when applied in the batter. Apparently moisture which is lost from the product during frying carries a substantially greater amount of water soluble antioxidants from the product when antioxidants are added in the batter than when they are sprayed onto the skin and covered with batter and breading. The mean diffusion path length for the antioxidant out of the product with the moisture is shorter when applied in the batter. Additionally, when antioxidants are deposited directly on the surface of the uncoated product, there is a greater opportunity for the water soluble antioxidants to preferentially diffuse into the high moisture interior rather than to diffuse out towards the incoming non-polar frying oil during cooking.

The degree of penetration of radioactive antioxidants into food products is an important consideration in the selection of delivery systems, and data for chicken legs are given in Tables 9 through 13. For ^{14}C-BHT (Table 9), only limited amounts penetrated to the muscle portion, and moderate amounts were observed in the skin layer. A penetration gradient relative to the method of application was observed for amounts of ^{14}C-BHT found in the skin layer. When sprayed onto the skin, 15.2% remained in that location, whereas slightly less (9.8%) was found in the skin when applied in the batter. Application through frying oil or spraying before freezing resulted in still lower concentration in the skin fractions (3.4 and 4.8%, respectively). It is noteworthy that very large percentages of the ^{14}C-BHT are located in the frying oil-rich batter and breading portion of the finished product. Similar penetration patterns in chicken legs were observed for ^{3}H-BHA (Table 10) and ^{3}H-PG (Table 11). The more water soluble ^{3}H-BHA and ^{3}H-PG show higher levels in the skin and muscle portions, and especially in the skin portion.

For penetration of the water soluble chelating-type antioxidants the necessity of application early in the process is readily evident in Tables 12 and 13. Very little penetration into the skin and muscle portions was observed for either ^{14}C-CA or ^{14}C-EDTA when applied by spraying before freezing. On the other hand, addition of either antioxidant through spraying before battering or with the batter resulted in extensive penetrations into the skin and muscle portions. Although not conclusive, data indicated that ^{14}C-CA penetrated into the muscle to a greater extent than the ^{14}C-EDTA. Relative diffusion rates of ^{14}C-CA and ^{14}C-EDTA could have been responsible for this difference. The stronger electrostatic attraction of ^{14}C-EDTA for polyvalent cations (19) in the tissue systems could have retarded its diffusion rate compared to that for ^{14}C-CA.

The ultimate resistance to oxidative flavor changes in breaded chicken legs depends on protection of susceptible lipids throughout the product (2). Thus, the distribution of antioxidants in the various lipid and non-lipid fractions according to location in the

product becomes a primary consideration. The overall distributions of radioactive antioxidants into these fractions based on the entire breaded chicken leg are presented in Table 14. The more complete distribution patterns based on the location of portions in breaded chicken legs are found in Tables 20 through 24. Comparisons for these data must be limited to those between processing methods for a single antioxidant. Valid intercomparisons between antioxidants cannot be made because of varying antioxidant polarities and subsequent partitioning effects exhibited for the various solvents employed in the extraction procedures.

In comparing the overall distribution data for ^{14}C-BHT (Table 14) it is readily evident that applying antioxidants before heat processing results in high amounts of the ^{14}C-BHT associating with the bound lipid fraction with some associations in the non-lipid fractions. On the contrary, application of ^{14}C-BHT during cooking in the frying oil does not result in a significant level of association of ^{14}C-BHT with the bound lipid fraction (Tables 14 and 20) nor does it result in appreciable penetration (Table 9). A similar distribution occurred when ^{14}C-BHT was applied by spraying after frying, but before freezing, demonstrating that heat alone does not cause associations of ^{14}C-BHT with bound lipid and non-lipid fractions. Further, a high percentage of ^{14}C-BHT is found in the batter and bread portion when applied by any of these methods. Therefore, it appears essential that the antioxidant be in close proximity to bound lipids and solid components under conditions that promote interactions leading to association of the antioxidant with these fractions. In the case where ^{14}C-BHT was added before frying, the system provides an opportunity for intimate association of food constituents and antioxidant in a high A_w environment prior to heating. Under these conditions the application of heat causes an interactive association between ^{14}C-BHT and the lipids, proteins, or starch of the bread and batter system (Table 20). On the other hand when ^{14}C-BHT is introduced via the frying oil, conditions are not conducive for formation of these associations. There are several factors that could contribute to this decreased interaction. Heating associated with frying causes progressive dehydration from the surface, and this is accompanied by oil replacing water, and possibly resulting in site inactivation due to denaturation, gelatinization, or marked decrease in A_w and overall polarity. Thus, ^{14}C-BHT carried with the oil arrives at the inactivated sites, and is unable to associate with them. Therefore, it remains dispersed in the free (non-polar) lipid phase.

The implications of antioxidant associations with bound lipids in preventing development of oxidative off-flavors in foods are profound. The bound lipid fraction contains polar lipids found in membranes of native and disrupted tissue systems, and phospholipids and proteins are major constituents of these structures (9). Antioxidant associations with membranes would place the antioxidants in close proximity to the highly unsaturated fatty acids of phospholipids. In the

analysis for distribution of antioxidants in fractions, chloroform-methanol should extract the antioxidants from the membrane systems. However, it is evident that some radioactivity is associated with the solid fractions (Table 20). This may reflect lipoprotein or protein-binding of antioxidants or perhaps antioxidant associations with starch structures.

In distribution studies (Table 14) where ^{3}H-BHA was applied to chicken legs by spraying before freezing and in frying oil, results similar to those for ^{14}C-BHT were obtained, i.e., most of the radioactive antioxidant was in the free lipid fraction. However, whereas ^{14}C-BHT applied by spraying before battering and in the batter followed by deep-frying resulted in significant associations with the bound lipid fraction, ^{3}H-BHA failed to associate with the bound lipids to the same extent in both instances. When ^{3}H-BHA was applied in the batter, some radioactivity was recovered from the bound lipid (15.3%), aqueous (11.6%), and solid (13.2%) fractions. However, very low amounts of ^{3}H-BHA radioactivity were recovered from these fractions when the antioxidant was applied by spraying before battering. Similar observations were made in corresponding studies with fish sticks (Table 15). It is interesting to note that ^{3}H-BHA penetrated to a greater extent in chicken legs (Table 10) than did ^{14}C-BHT in the same product (Table 9), possibly because of its smaller molecular size and greater volatility. Although ^{3}H-BHA tended to penetrate more readily into the skin portion, it did not associate with the bound lipid fraction (Table 21). Based on the physical and chemical properties of ^{3}H-BHA compared to ^{14}C-BHT, a rationale for the lack of association of ^{3}H-BHA with bound lipids is not evident. Unexplained synergistic effects of BHA and BHT in foods have long been known (4), and based on the current study, it would appear that synergistic protection may involve protection of specific lipid fractions by each antioxidant. However, if extracting solvent polarities have not influenced the associative distributions, the roles of BHA and BHT appear reversed from those anticipated when polarity considerations form the basis for the protective lipid associations.

Application of ^{3}H-BHA to breaded chicken legs by immersion freezing in Freon^R Food Freezant resulted in unexpected and extensive penetration (Table 10) into the muscle portion (58.6%), but only a small amount remained in the skin portion (7.9%). When the distribution data for ^{3}H-BHA in the various fractions (Table 14) of the overall chicken leg are examined, another unusual observation can be made in that an exceptionally large amount (31.9%) of the radioactivity resides in the aqueous fraction (Table 21) and was associated with the muscle portion (23.8%). In comparison only 6% was found in the batter and bread portion and only 2.1% in the skin fraction. The data on penetration and association with various fractions for chicken legs (Tables 10 and 14, respectively) contrast markedly with the data for fish sticks (Tables 10 and 15). However, the distribution pattern for associstion of ^{3}H-BHA with various fractions in frankfurters

(Table 16), beef stew (Table 18), carrots (Table 19) and pork chops (Table 17) were similar to that observed for chicken legs. In looking for an explanation of these data, it was observed that immersion freezing applications of ^{3}H-BHA to each of the food systems except fish sticks were completed late in the study. For the fish sticks, the immersion freezing application was completed earlier in the experimental design to determine feasibility of adding radioactive antioxidant by this method. Since the ^{3}H-BHA was stored in an ethanol solution after initial purification, there may have been some tritium exchange between ^{3}H-BHA and ethanol prior to use with fish sticks, but there would undoubtedly be much more tritium exchange prior to use with the other five products. Thus, it would appear that the most plausible explanation for the high association of radioactivity with the aqueous fraction in chicken legs, frankfurters, beef stew, carrots and pork chops is due to ^{3}H-ethanol and not ^{3}H-BHA. The presence of a significant quantity of ^{3}H-ethanol would also account for the good penetration into the interior of chicken legs and frankfurters. The data for fish sticks indicate a more reasonable distribution and probably represent ^{3}H-BHA and not ^{3}H-ethanol distributions. A further confirmation that the distribution into various fractions was influenced by ^{3}H-ethanol was an isolated experiment with ^{14}C-BHT delivered to carrots by immersion freezing (Table 19). Since there would be no radioactivity exchange between ^{14}C-BHT and components in its environment, it would be anticipated that little radioactivity would be found in the aqueous fraction, and experimental data (Table 19) confirm that anticipation. Thus, interpretation of all of the data on ^{3}H-BHA added by immersion freezing must be done with extreme caution. It is possible that other explanations can be developed which will reconcile all of the data, but in any event further experimental work is necessary.

Introduction of ^{3}H-PG into chicken legs by the various methods (Table 11) showed limited mobility and penetration in spite of the polar nature of PG and its theoretical potential to diffuse through the continuous aqueous phase. The effect of heat in redistributing ^{3}H-PG in the chicken legs can be seen when the radioactivity data for spraying before battering and spraying before freezing are compared. The diffusion path length effect on penetration can be readily seen by comparing radioactivity data for spraying before batter and adding with batter. The appreciable water solubility of ^{3}H-PG is reflected in data obtained for its distribution in fractions of chicken legs (Table 14). It is noteworthy that ^{3}H-PG was generally found in substantial amounts in each of the fractions. Whereas the amount of ^{14}C-BHT found in the bound lipid fraction was increased dramatically by heating, heat had limited influences on the distribution of ^{3}H-PG (Table 14). The data for ^{3}H-PG distribution in the various portions of chicken legs (Tables 11 and 22) show that it tends to remain at the site of application and does not migrate into the batter and bread from the skin as readily as ^{14}C-BHT (Table 9) and ^{3}H-BHA (Table 10) which are more lipid soluble.

Distribution data for ^{14}C-CA and ^{14}C-EDTA in chicken legs (Tables 14, 23 and 24) show that nearly all of the radioactivity is found in the aqueous and solid fractions with at least 65% of the total being in the aqueous fraction. That radioactivity remaining in the solid fraction should be electrostatically associated with proteins and other immobile cationic constituents. As previously mentioned for ^{14}C-CA and ^{14}C-EDTA, the processing method influences the retention (Table 3) and penetration into various portions of chicken legs (Tables 12 and 13), but the processing method does not appear to influence the distribution of ^{14}C-CA and ^{14}C-EDTA into various fractions (Tables 23 and 24).

For the subsequent storage stability studies of breaded chicken legs, the selection of the antioxidant and delivery system was based on the foregoing data, and also on considerations for the inclusion of each antioxidant into a food system. Since the high unsaturated fatty acid content of the phospholipid fraction of chicken has been considered a major factor in the development of oxidized off-flavors (2), the choice of delivery method and antioxidant was based on achieving a significant antioxidant association with the bound lipid fraction. BHT was chosen for the free-radical terminator because of its bound lipid association (Table 14) and its high retention through processing (Table 3). The chelating-type antioxidant selected was EDTA, and application by spraying before battering was chosen for both antioxidants.

Data for sensory evaluation of breaded chicken legs during 6 mo frozen storage (-26 C) are presented in Figs. 2, 3 and 4. The overall acceptability scores for chicken legs both with and without antioxidants (Fig. 2) remained high and essentially constant. The slightly higher rating for the commercial reference samples probably reflects the absence of the warmed-over flavor, whereas, the frozen, stored samples both exhibited this flavor. Warmed-over flavor has been equated by some with the development of oxidized flavor in precooked meats (20). however, in all cases a direct relationship between warmed-over flavor and lipid oxidation has not been thoroughly established. In view of the complex nature of chicken aroma volatiles, some of the warmed-over flavor may be due to development of flavors from components other than lipids and may be sulfur-containing compounds.

The intensity of oxidized flsvor (Fig. 3) and other off-flavors (Fig. 4) remained low throughout the storage period. The overall sensory quality of frozen chicken legs during the 6 mo storage period was such that the product remained very acceptable.

The results of chemical stability tests for chicken legs are given in Figs. 5, 6, 7 and 8. The TBA values (Fig. 5) remained low (< 1.0) throughout the storage period, and differences between the samples were inconsistent and show no definite trends. Ultraviolet

absorbances at 232 and 268 nm for lipids extracted from the frozen, stored chicken legs (Figs. 7 and 8, respectively) consistently show slightly higher values for the samples without antioxidants. However, the magnitudes of the values remain essentially constant throughout the storage period. Peroxide values (Fig. 6) were extremely low (< 5) and did not indicate a progression of lipid oxidation. Again there was essentially no difference between samples with or without antioxidants. Attempts to chemically measure BHT and EDTA in the breaded frozen chicken legs were unsuccessful because of substances from the chicken that interferred with the color determination.

In summary, in this study, precooked, breaded, frozen chicken legs were found to be remarkably stable to the development of oxidized flavor during 6 mo storage regardless of the presence of added antioxidants. Both the sensory evaluation and chemical analyses data support this conclusion. Extension of storage periods beyond 6 mo might provide useful information about potential antioxidant systems for breaded chicken legs. Practical guidelines for application of antioxidants by methods used in this study are summarized in Table 35. The amount of each antioxidant in each delivery system is based upon the retention of the antioxidant through processing so that a final concentration of 200 ppm (fat basis) is achieved in the product.

B. Introduction of Antioxidants into Fish Sticks.

Breaded, precooked fish sticks were prepared from frozen blocks of Atlantic Cod (*Gadus morhus*) because fresh fish fillets were unavailable locally. Introduction of antioxidants into commercial quality fish products during the latter stages of processing does not achieve maximum protection, but provides a model for studying the behavior of various antioxidants in a frozen product sensitive to lipid oxidation. Data obtained from the methods of application studied will provide information for the development of controlled antioxidant application technology in the seafoods processing industry. The finished fish sticks contained the following fat levels (wet weight): overall, 7.4%; breading, 10.9%; outer layer, 2.0%; and center portion, 0.4%. The antioxidant delivery systems investigated are summarized in Table 1, and included spraying before battering, addition in the batter, during cooking, in the frying oil, spraying before freezing, and immersion Freon^R Food Freezant freezing.

The data showing retention of radioactive antioxidants through processing based on the amounts applied initially and that remaining in the finished breaded fish sticks are presented in Table 4. Based on the retention results, practical amounts of antioxidants for each delivery system were calculated to yield 200 ppm (fat basis) of each antioxidant in the finished product, and these are given in Table 35. Complete retention of antioxidants is indicated for spraying before freezing, application in frying oil, and immersion freezing because no further processing steps contributing to losses of antioxidants were involved.

Similar to breaded chicken legs, ^{14}C-BHT was nearly all retained through the processing methods employed. Nearly half of the more volatile ^{3}H-BHA was lost when applied by either spraying before battering or by adding with the batter. ^{3}H-PG and ^{3}H-BHA were retained to nearly the same extent in fish sticks as in chicken legs, except that slightly higher retentions of ^{3}H-BHA were noted in the fish sticks (Table 4). Therefore, for the same rationale as presented for chicken legs, the primary route for antioxidant losses in deep-fried, breaded meat and fish products appears to be direct volatilization and steam distillation, and only secondarily by extraction into the frying oil.

For the water soluble, chelating-type antioxidants, ^{14}C-CA and ^{14}C-EDTA, slightly greater losses were experienced with fish sticks than with chicken legs when applied by either spraying before battering or with the batter. This may be the result of higher moisture contents of the fish stick portions (i.e., moisture in fish sticks: breading, 42.5%; outer layer, 74.5%; and center portion, 77.9%; compared to chicken legs: breading, 36.4%; skin, 42.1%; and muscle portion, 71.2%). Moisture emerging from the interior of the fish sticks would be expected to carry water soluble antioxidants toward the surface of the frying product, possibly to a greater extent than for chicken legs.

The data for the degree of penetration of radioactive antioxidants into fish sticks are presented in Tables 9 through 13. For ^{14}C-BHT (Table 9) and ^{3}H-BHA (Table 10) it can be seen that when the antioxidants are applied prior to the frying steps, penetration into the product is greatly enhanced. Although limited, penetration of ^{3}H-BHA into the center portion of fish sticks was greater than for ^{14}C-BHT, possibly because of volatility and polarity considerations.

In essence, however, the ^{3}H-BHA and ^{14}C-BHT penetration data for fish sticks and chicken legs were complementary, and the antioxidants showed similar behavior in the two food systems.

Application of ^{3}H-BHA by immersion freezing resulted in limited penetration into the fish sticks contrasting to the data for other products. As discussed in the section on chicken legs, the data for penetration and distribution into various fractions for fish sticks when ^{3}H-BHA is added by immersion freezing probably represent the most accurate data since there was least opportunity for tritium exchange between ^{3}H-BHA and ethanol. The data on fish sticks indicate that this method of addition when products are dipped or sprayed with Freon^{R} Food Freezant is worthy of further consideration and investigation.

Data for ^{3}H-PG in fish sticks (Table 11) showed a general penetration pattern similar to that for chicken legs, except that when it is applied by spraying before battering a greater migration of ^{3}H-PG into the breading was observed. This transfer probably reflects a

greater mobility of ^{3}H-PG along with interior moisture leaving the product. When ^{3}H-PG was applied in the batter, the ^{3}H-PG showed limited mobility because the batter was dehydrated to a greater extent in the early stages of frying, and remained in situ.

The data for ^{14}C-CA and ^{14}C-EDTA in fish sticks (Tables 12 and 13, respectively) showed that these antioxidants penetrated the product to similar extents; these data paralleled that observed for chicken legs. Again, heat processing after antioxidant application results in enhanced penetration, particularly when antioxidants are added by spraying before battering.

The distribution of antioxidants into various fractions of entire fish sticks is summarized in Table 15. The more complete distribution patterns based on the location of portions in breaded fish sticks are found in Tables 25 through 29. Comparisons for these data are necessarily limited to processing methods for single antioxidants because of partitioning effects due to varying polarities of extracting solvents. In comparing the overall distribution data for ^{14}C-BHT (Table 15), it can be seen that antioxidant application prior to heat processing yields a significant association with the bound lipid fraction. As with chicken legs, application of ^{14}C-BHT in the frying oil does not result in its association with the bound lipid fraction to any appreciable extent. Spraying ^{14}C-BHT before freezing also resulted in low association with the bound lipid fraction. The same reasoning as discussed for chicken legs would appear to apply for fish sticks.

Generally ^{3}H-BHA was found in the free lipid fraction (Table 15). When ^{3}H-BHA was applied in the batter some radioactivity was recovered from the bound lipid (10.5%), aqueous (9.8%), and solid (12.8%) fractions and this was similar to that reported for chicken legs. In the case of spraying before battering, ^{3}H-BHA was present to a greater extent in the outer portion of the fish stick (Table 26) than ^{14}C-BHT applied in a similar manner (Table 25). This may indicate that ^{3}H-BHA was less readily transferred from the site of application into the breading, possibly as a result of polarity.

Unlike application of ^{3}H-BHA by immersion freezing to other products, little radioactivity was found in the aqueous fraction. As discussed previously, the distribution of ^{3}H-BHA into various fractions in fish sticks probably represents the true distribution since it was influenced less by presence of ^{3}H-ethanol.

The data for ^{3}H-PG distribution in fish sticks indicates that a substantial amount of radioactivity ($>$30% of the total) was located in the solid fraction regardless of the method of application (Table 15). Further, this same characteristic was observed in each fish stick portion analyzed (Table 27). On the other hand, much smaller amounts

of ^{3}H-PG were found in the solid fractions from the chicken. The reason for the lower amounta observed in the chicken legs solid fractions is not clear, but the ready loss of moisture from fish proteins (21) may create a suitable environment for substantial associations between ^{3}H-PG and partially dehydrated proteins. Also, chicken breading and skin portions showed higher fat levels (19.7 and 22.6%, respectively) than did correaponding portions for fish sticks (10.9 and 2.0%, respectively). The relative amount of free lipid may have influenced the distribution of the relatively polar ^{3}H-PG.

All of the ^{14}C-CA and ^{14}C-EDTA applied by the various methods was found in the aqueous and solid fractions of fish sticks. The overall distribution data for both ^{14}C-CA and ^{14}C-EDTA in fish sticks (Table 15) seems to indicate less association between the antioxidants and the components of the solid fraction than found for chicken legs (Table 14). In evaluating the detailed distribution data for ^{14}C-CA in fish sticks (Table 28) compared to ^{14}C-CA in chicken legs (Table 23), it can be seen that the potentially more dehydrated proteins of fish muscle associate much less readily with ^{14}C-CA (c.f., 2.3% of total for batter application) than the proteins of chicken skin (c.f., 9.4% of total for batter application). The same observations can be made for similar ^{14}C-EDTA applications to fish sticks (Table 29) and chicken legs (Table 24).

For the storage stability studies BHA was selected as the free radical terminator antioxidant to incorporate BHA into the experimental design, and CA was chosen for a similar reason. Both antioxidants were applied by spraying before battering to achieve intimate contact with fish muscle before heat processing. The sensory evaluation data for breaded fish sticks during 6 mo storage at -26 C are summarized in Figs. 9, 10 and 11 and the data for chemical analyses during storage are summarized in Figs. 12, 13, 14 and 15. The overall acceptability scores of fish sticks declined slightly during 6 mo storage (Fig. 9) and correspondingly the relative intensity of oxidized flavor increased slightly (Fig. 10). The overall acceptability scores for the commercial reference samples employed also decreased slightly but did not show a corresponding increase in oxidized flavor intensity. This may be due to changing commercial quality or a subtle panel awareness of other fish quality attributes. However, the intensity scores for other off-flavors remain relatively constant throughout the study (Fig. 11). It is noteworthy that the application of BHA and CA did not exhibit any protective effect during 6 mo storage at -26 C but all samples were still considered acceptable after this storage period.

TBA numbers and peroxide values remained essentially constant throughout the study and showed no differentiation between the sample with BHA and CA and the control sample (Figs. 12 and 13, respectively). UV absorbances were quite constant throughout; however, the sample without antioxidants showed slight but consistently higher values at both 232 and 268 nm (Figs. 14 and 15, respectively). These data

indicate a slightly faster initial rate of lipid peroxidation in the sample without antioxidants.

The data for chemical analysis for BHA in fish sticks are summarized in Table 40. This was the only successful chemical measurement of an added antioxidant in the storage stability studies. Even so, the recovery for freshly added BHA through the method was only about 21% and variability in the method was about $\pm$ 20%. The data in Table 40 indicate that approximately 100 ppm of measurable (active) BHA was present immediately after an intended application of 150 ppm on a fat basis and this level was maintained throughout the storage period.

C. Introduction of Antioxidants into Frankfurters.

Frankfurters were chosen as precooked high fat (25%), comminuted meat products that are susceptible to oxidation during frozen storage. The methods of introduction are summarized in Table 1, and these were all by direct addition at different stages of frankfurter manufacture except in the case of ^{3}H-BHA application by immersion Freon^{R} Food Freezant freezing. Similarities in the direct addition methods for antioxidant application provided an opportunity for assessing the reproducibility of the radioactive assays and chemical analyses employed in this study.

The retention data for radioactive antioxidants in frankfurters are given in Table 5, and practical incorporation data to achieve 200 ppm of antioxidants on a fat basis are given in Table 35. It can be seen in Table 5 that the retention of all antioxidants was very high through the processes evaluated, and this can be attributed in part to the thorough incorporation of the antioxidants throughout the frankfurters. The phenolic antioxidant retentions were similar and were all greater than 88 percent. The retentions for the water soluble chelating-type antioxidants, ^{14}C-CA and ^{14}C-EDTA, were similar to each other, but were slightly lower than that observed for the phenolic antioxidants. This probably can be attributed to their solubility in the drip loss fraction during cooking. Within an antioxidant series, it appears that the time of addition of the antioxidant does not alter the retention of that antioxidant. Also, the data collected for antioxidant additions within a series reflect the replication of the incorporation and analysis procedures. As with other products, 100 percent retention of ^{3}H-BHA is shown for application by immersion Freon^{R} Food Freezant freezing because there are no subsequent opportunities for loss. The location data for radioactive antioxidants in the outer layer, intermediate layer, and center portion of frankfurters are summarized in Tables 9 through 13. The only diffusion controlled penetration involved in the frankfurter study was in the instance of ^{3}H-BHA application by immersion freezing (Table 10). Data for the direct addition application methods show that the distribution by location for all of the antioxidants in the processed frankfurters were very similar (range 24.8 to 41.0; mean. 33.3 percent of total

radioactivity). This indicates that neither the chemical or physical antioxidant characteristics nor the processing factors (e.g., drip losses) significantly affect the final equilibrium concentration of antioxidants throughout the product. Consequently, concentration gradients in the products were not observed.

In the case of immersion freezing, ^{3}H-BHA was delivered in a manner that resulted in location dependent concentrations (Table 10) within the frankfurters but, as discussed for chicken legs, this probably reflects ^{3}H-ethanol distribution, not ^{3}H-BHA.

The data for overall distribution of radioactive antioxidants into various fractions of frankfurters are presented in Table 16 and the detailed distribution data according to locations are given in Tables 30 through 34. In overall evaluation of the direct addition data, it can be seen that within each antioxidant series good replication was achieved. The only exception occurred in the instance of ^{3}H-BHA addition during mixing where a significant amount of radioactivity was found in the solid fraction (Table 16). Interestingly, the distribution according to location in the frankfurters was consistent for each portion (Table 31, outer, intermediate and center) indicating some exceptional interaction as a result of that particular method of incorporation.

In general all of the phenolic antioxidants applied by direct addition exhibited notably high associations with the bound lipid fraction. This is in contrast to observations made for the other food products where concentrations of ^{3}H-BHA were generally low in the bound lipid fraction. Significant amounts of ^{14}C-BHT, on the other hand, were generally found in the bound lipid fraction of those foods receiving substantial heat treatment after antioxidant application (c.f. Tables 14 and 15). The distribution data for all phenolic antioxidants appear to substantiate the hypothesis that high A_w conditions, elevated temperatures, and close proximity of antioxidant and reactive site all promote the association with the solid fraction of frankfurters, and similar observations were made for fish sticks (Table 15). ^{14}C-CA and ^{14}C-EDTA behaved similarly in frankfurters, and all radioactivity for each was found in the aqueous and solid fractions and this was similar to that observed for fish sticks (Table 15) and chicken legs (Table 14).

For the storage stability studies BHT was selected as the free radical terminator antioxidant because of its high association with the bound lipid fraction (Table 16) and it was added during mixing. EDTA was selected as the chelating-type antioxidant to provide an opportunity for its evaluation even though both ^{14}C-CA and ^{14}C-EDTA behaved similarly in frankfurters. EDTA was added during chopping. The sensory evaluation data for frankfurters during 6 mo storage at -26 C are summarized in Figs. 16, 17 and 18, and the data for the chemical analyses during storage are summarized in Figs. 19, 20, 21

and 22. It can be seen (Fig. 16) that the overall acceptability of both frankfurters with and without antioxidants decreased during the 6 mo storage period, but that the rate of decline was less for the sample containing BHT and EDTA. In Fig. 17 it can be seen that the degree of oxidized flavor development was greater in mid-storage periods for the sample without BHT and EDTA; but that after 6 mo the intensity of oxidized flavor was the same for both products. The high overall acceptability (Fig. 16) and the low oxidized flavor scores (Fig. 17) for the commercial reference samples compared to the experimental sample scores probably reflect less technological process control in the preparation of the experimental frankfurters. The relative off-flavor intensity scores for frankfurters (Fig. 18) show some differences between the commercial and the experimental, with and without antioxidant, samples. However, these scores remained low and constant throughout the test period and probably reflect judge confusion-error related to the presence of smoke and oxidized flavors.

The TBA value data (Fig. 19) for experimental frankfurters supported the taste panel findings in that the samples with antioxidants exhibited a delay in the onset of oxidation, but after 6 mo little difference was noted. The peroxide values (Fig. 20), on the other hand, were low for both samples throughout the study, and did not exhibit a discernible pattern. Similarly, the UV absorbance data at 232 nm (Fig. 21) and 268 nm (Fig. 22) did not vary through the study, and did not show any response to the presence of BHT and EDTA. Analyses for BHT in frankfurters at various intervals during storage were unsuccessful because none was detected. In the case of EDTA interferring absorbances in the colorimetric determination prevented analysis.

The overall storage stability data indicated that BHT and EDTA incorporated by direct addition to the ingredients delays the development of pronounced oxidized flavors, and after 6 mo storage at -26 C the protective effect is overwhelmed. Although the overall acceptability scores (Fig. 16) for frankfurters were quite low (3.5-4.0) after 6 mo, the relative palatability of these frankfurters was greater than that observed for any of the freeze-dried products after about 2 mo storage at 32 C.

D. Introduction of Antioxidants into Freeze-Dried Pork Chops.

Freeze-dried lean pork chop pieces (6.9% fat wet weight, cooked) were selected for study because they are highly susceptible to lipid oxidation and are representative of low A_w meat products. The methods of antioxidant application (Table 1) were chosen because they were either easily implemented (dipping before and after steam precooking) or were innovative, exploratory techniques (immersion Freon[R] Food Freezant freezing, or freeze-dry vacuum release).

The data obtained for the retention of radioactive antioxidants are presented in Table 6. It is notable that a major portion (ca 80%) of the phenolic antioxidants ^{14}C-BHT, ^{3}H-BHA, and ^{3}H-PG) were retained when added by dipping either before or after precooking. No selective losses of phenolic antioxidants from pork pieces were observed that could be attributed to fundamental molecular properties. Since the retained levels of phenolic antioxidants were comparable and independent of precooking in the process sequence, it would appear that the freeze-drying step probably accounts for the losses through volatization. On the other hand, addition of the water soluble chelating-type antioxidants (^{14}C-CA and ^{14}C-EDTA) after cooking resulted in significantly higher retentions than when added by dipping before cooking. The greater losses were probably the results of drip losses during steam cooking which carried the readily water soluble antioxidants out of the product.

Application of ^{3}H-BHA by vacuum release following freeze-drying obviously resulted in complete retention since no subsequent process was employed. On the other hand, it was extremely difficult to control the level of antioxidant which was transferred to the pork pieces (Table 35, pork chops). ^{3}H-BHA was readily transferred by this technique, but considerable developmental technology would be required to achieve a practical system. The condition for application by freeze-dry vacuum release is also shown in Table 35, and further in the same table, this can be compared to other practical amounts of antioxidants required for achieving desirable levels with other methods of application.

Since the pork chops pieces in the model system employed were too small for physical sectioning, penetration studies were not attempted. Therefore, the distribution of radioactive antioxidants into the various fractions of pork is given only for the entire composite sample pieces. For ^{14}C-BHT, it can be seen in Table 17 that heating application again significantly increased the association between ^{14}C-BHT and the bound lipid fraction at the expense of the free lipid fraction. Heating after application did not noticeably affect the distribution of ^{3}H-BHA, ^{3}H-PG, ^{14}C-CA or ^{14}C-EDTA. The amount of ^{3}H-BHA found in aqueous fraction of pork (ca 18% of total) is somewhat elevated compared to that found for chicken legs, fish sticks or frankfurters ($<11\%$, c.f., Tables 14, 15 and 16, respectively). And again, very little ^{3}H-BHA in pork was found in the bound lipid fraction ($< 3.0\%$, Table 17). It can also be seen in Table 17 that very low levels of ^{3}H-PG were found in the free lipid fraction of pork ($< 2.5\%$ of total) in particular contrast to chicken legs where up to about 30% of the radioactivity was located in the free lipid fraction (Table 14). This may indicate a very low level of non-polar lipids in precooked pork as compared to breaded chicken legs.

Delivery of ^{3}H-BHA by freeze-dry vacuum release gives a distribution pattern similar to that observed when applied by dipping.

The water soluble chelating-type antioxidants ^{14}C-CA and ^{14}C-EDTA were found concentrated in the aqueous and solid fractions similar to that observed for all other products. The distributions of these two antioxidants in the fractions of pork do not appear significantly influenced by processing imposed.

The storage stability study for freeze-dried pork chops employed application of BHA by immersion freezing in Freon[R] Food Freezant because this was a new, innovative method and this would demonstrate effectiveness of application by this method. For the water soluble chelating-type antioxidant, CA was applied by dipping after precooking to achieve high retention. The sensory evaluation data for the stored freeze-dried pork chops are shown in Figs. 23 through 25, and the chemical analysis data are given in Figs. 26 through 29. The general quality of the freeze-dried pork chops was low and the overall acceptability scores (Fig. 23) compared to fresh pork chops illustrate this point. The low apparent quality was due to poor rehydration characteristics and the rapid onset of oxidative flavor deterioration (Fig. 24). The product quality deteriorated so rapidly that the sensory evaluations were terminated after 4 mo storage at 32 C. The decline of overall acceptability scores (Fig. 23), the development of oxidized flavor (Fig. 24), and the general increase in other off-flavors (Fig. 25) show definite linear trends, but the antioxidant system was totally ineffective in protecting the sensory quality of pork chops. The ineffectiveness of the antioxidant system is also illustrated in the data for the chemical tests (Figs. 26 through 29). The peroxide values (Fig. 27) were high initially indicating rapid onset of oxidation in both samples, and then declined during the storage period. The TBA numbers (Fig. 26) and the UV absorbances (Figs. 28 and 29) remained essentially constant throughout the storage. Attempted analysis for levels of BHA in pork chops after various storage periods were unsuccessful because of interferences in the colorimetric portion of the procedure.

E. Introduction of Antioxidants into Freeze-Dried Beef Stew.

Freeze-dried beef stew was selected for inclusion in this study because it is a combination of several tissue (animal and plant) and homogeneous food components. Previous reports (2) have indicated the extreme susceptibility of this product to oxidative deterioration.

The methods of antioxidant application for beef stew (Table 1) were chosen for compatibility with normal preparation or to examine innovative, new methods of application. The retentions of various radioactive antioxidants through preparation of freeze-dried beef stew are summarized in Table 7. It is noticeable that the application by direct addition and dipping resulted in very similar retentions and the retentions were less than those observed for all other products except carrots. Some losses of the phenolic antioxidants, especially the more volatile ^{3}H-BHA and ^{14}C-BHT, would be anticipated because of

the freeze-drying process. However, the substantial loss of the water soluble and nonvolatile ^{14}C CA and ^{14}C-EDTA indicate that some other mechsnism responsible for st least a portion of the overall losses is involved. In reviewing the process thera appears to be little opportunity for loss or destruction, but inability to completely recover residue from the freeze-drying trays is believed to be largely responsible. If this is the mechanism, then the antioxidants appear to have been concentrated at the product-container interface. Further evidence for involvement of unusual loss mechanism can be seen by examining the retention data for immersion freezing of beef stew (Table 7). If volatilization during freeze-drying were a msjor factor in the losses, then the immersion frozen product should have behaved similarly. Again as with other products application by freeze-dry vacuum release resulted in complete retention because the labeled antioxidant is added at the end of the entire process.

Since the stew pieces were small, penetration studies were not attempted. The distributions of the rsdioactive antioxidants in various fractions of beef stew are summarized in Table 18. The data for ^{14}C-BHT added by both methods show similar distribution patterns except a slightly higher amount of radioactivity was found in the solid frsction when incorporated with the ingredients. Minimal amounts of ^{14}C-BHT were found in the bound lipid fraction (ca 10%). Considering all the ^{14}C-BHT distribution data in this study it can be seen that the lowest level of ^{14}C-BHT found in a bound lipid fraction was about 5%, and this probably reflects the partitioning snd solubility-limit effects in hexane. The dsta for ^{14}C-BHT in Table 18 indicate then that slight association with the bound lipid fraction may have occurred during heating, but the amount is dramatically less than thst observed for other foods (c.f. Tables 15 snd 16).

For ^{3}H-BHA, there was an unexplained dissimilarity between the amount of ^{3}H-BHA found in the bound lipid fraction when added by direct addition or by dipping ingredients. The high level found in the sample where ^{3}H-BHA wss sdded by dipping before cooking is difficult to explsin and would appear to be experimental error. When ^{3}H-BHA was sdded with ingredients, a distribution pattern similar to that observed in many other products was obtained. For immersion freezing, the distribution probably reflects ^{3}H-ethsnol distribution, not ^{3}H-BHA, as discussed for chicken legs.

In the case of ^{3}H-PG, the two methods of application yielded similar distribution patterns and substantial amounts of radioactivity was found in the free lipid, bound lipid and aqueous fractions. Very little was found in the solid fraction contrary to that observed for high A_w products. The low smounts of ^{3}H-PG associated with the solid frsction in sll of the low A_w products may be the result of disrupting the associative mechanisms by moisture removal. On the other hand, this may also be a result of simple ^{3}H-PG partitioning between the extracting solvents and the milieu of the high and low A_w food products.

For the high A_w products, the ^{3}H-PG may be partitioned in the extracting solvent to a lesser extent than for low A_w products.

For the water soluble, chelating-type antioxidants, ^{14}C-CA and ^{14}C-EDTA, similar patterns were obtained in each instance. It is noteworthy that low amounts of ^{14}C-CA and ^{14}C-EDTA were found in the solid fractions, an observation made only for beef stew and fish sticks. Similarities or dissimilarities between composition of all products does not readily yield an explanation for these observations.

Based on the retention data, practical amounts of each antioxidant to incorporate into the delivery system to achieve 200 ppm on a fat basis is given in Table 35. Application of sufficient amounts of ^{3}H-BHA by freeze-dry vacuum release was difficult to achieve because of the quantity required to yield 200 ppm on a fat basis (1.3% fat). Also, as observed for other products, reproducibility of application was not attained.

For the storage stability studies, BHA and CA were incorporated in the beef stew by direct addition in the ingredients. These antioxidants and method of application were chosen to complement the overall experimental design, and to provide intimate antioxidant-food product contact. Sensory evaluation data are summarized in Figs. 30, 31 and 32, and the chemical stability data are summarized in Figs. 33, 34, 35 and 36.

The overall acceptability scores (Fig. 30) show an extremely rapid decline to a level such that sensory evaluation was terminated after four months. The incorporation of a calculated 150 ppm BHA and CA had essentially no influence on the storage stability. If it is assumed that a mean overall acceptability score of 4.0 represents the cutoff point for acceptability, then the product was suitable for one month when stored at 32 C in air. This is in agreement with data cited by Labuza (2).

The decline in overall acceptability was paralleled by an increase in relative oxidized flavor intensity (Fig. 31). In Fig. 32, it can be seen that the panel data showed an increase in the relative intensity of other off-flavors. This was attributed to the concurrent presence of typical oxidative rancidity flavors and a distinct, sharp hexenal-like aroma. The hexenal would have been derived from oxidative processes of lipids but is not normally associated with classical rancidity notes.

The TBA values (Fig. 33) for the beef stew without antioxidant were consistently slightly higher than for beef stew with antioxidants but, contrary to expectations, values declined during storage. In Fig. 34, the peroxide values for the sample with antioxidants were generally higher than those for samples without BHA and CA. While higher peroxide values might be anticipated for the sample without

antioxidants, the lower values observed for this sample may reflect more rapid hydroperoxide degradation to secondary products, such as aldehydes. There also appears to be two distinct phases of hydroperoxidation which could reflect the oxidation of two different types of lipids.

UV absorbances (Figs. 35 and 36) show consistently higher values for samples without antioxidants indicating that presence of antioxidant inhibits the hydroperoxide formation and subsequent shifts to conjugated systems.

The antioxidant systems employed (concentration and types) were inadequate for protection of beef stew when stored at 32 C in air which is fundamentally in agreement with Pintauro (22) and the data cited by Labuza (2). Chemical analysis for BHA were unsuccessful because of TLC and colorimetric interference coupled with extremely low recovery.

F. Introduction of Antioxidants into Freeze-Dried Carrots.

Freeze-dried carrots were chosen for study because they are representative of low A_w foods containing high levels of β-carotene [about 11,000 IU/100 gm, USDA Handbook No. 8, (7)] that are extremely susceptible to oxidative deterioration. The methods of antioxidant application chosen for this study are summarized in Table 1, and included application by spraying before freezing and three new, innovative methods. The retention data for radioactive antioxidants through preparation of freeze-dried carrots are given in Table 8. Application of the phenolic antioxidants by spraying before freezing resulted in significant losses through processing. As discussed for freeze-dried beef stew, the losses may be attributed in part to volatilization losses during freeze-drying. The water soluble, chelating-type antioxidants, ^{14}C-CA and ^{14}C-EDTA, were retained to a much higher degree than phenolic antioxidants indicating that volatilization was probably a significant loss mechanism. However, since some ^{14}C-EDTA was lost when applied by spraying before freezing, some losses might be attributed to incomplete recovery of carrot solids from the freeze-drying tray after freeze-drying. This loss mechanism was also proposed to account for losses from freeze-dried beef stew. For ^{14}C-CA, its solubility and its smaller molecular size which may result in more rapid diffusion may account for its excellent retention. Further, evidence for loss of ^{14}C-BHT by volatilization during the freeze-drying operation can be seen by examining the retention data for application by immersion freezing. A very significant loss was experienced. The poor retention of ^{14}C-BHT during freeze-drying may be a result of the quantity and type of lipid present in carrot tissue. For purposes of this study, the antioxidant was added on the basis of achieving association of the antioxidant with β-carotene. Since β-carotene is distributed throughout the carrot tissue and the antioxidants are applied only on the surface, there would be little opportunity to relocate the antioxidant to the site of the β-carotene

except by diffusion. Since the temperature of the product after application would be below freezing almost immediately in all cases, diffusion would be limited and the antioxidant would be susceptible to volatilization during freeze-drying.

Application of ^{3}H-BHA in steam during steam blanching resulted in a significantly greater retention of the antioxidant when compared to other methods of adding ^{3}H-BHA prior to freeze-drying. This was probably due to greater penetration of the antioxidant into the carrot pieces as a result of being applied in the vapor state. Since the carrot pieces were too small for sectioning, the penetration of the antioxidant into the piece could not be measured. However, it would appear that this method is worthy of further study.

As with other products, application of radioactive antioxidants by freeze-dry vacuum release resulted in 100% retention. Practical levels of antioxidants in the various delivery system to achieve 200 ppm, β-carotene basis, are presented in Table 35. On freeze-dried carrots, freeze-dry vacuum release methodology was tested. There was considerable variation in the reproducibility of the method and the temperature of the antioxidant significantly influenced the delivered amount. Increased temperatures (up to 100 C) were necessary to mobilize quantities of ^{3}H-BHA from the stock supply to provide at least 200 ppm for the fat phase. Both ^{3}H-BHA and ^{14}C-BHT could be applied by this method. However, because of the low absolute amounts of antioxidant transferred, this system would probably only be feasible with products of very low fat content, such as dried fruits or vegetables. For that application, it would appear that developmental technology is worthy of further investigation.

As pointed out for the pork chop pieces and beef stew, penetration studies were not conducted on carrot pieces because of their small size. However, distribution of radioactive antioxidants into the various fractions was accomplished and the data are presented in Table 19. When ^{14}C-BHT was added by spraying before freezing there was a significant association with the bound lipid fraction, whereas when ^{14}C-BHT was added by immersion freezing or freeze-dry vacuum release, there was little association. There does not appear to be an obvious explanation for this difference since there were no significant differences in treatment.

With ^{3}H-BHA, application by steam blanching and immersion freezing resulted in a significant association with the aqueous fraction and application by immersion freezing in Freon^{R} Food Freezant resulted in significant association in the bound lipid fraction. The association of ^{3}H-BHA with the aqueous fraction when added during steam blanching may possibly be attributed to the effect of heat on tritium exchange between ^{3}H-BHA and other food constituents or water (steam). For the immersion freezing, the distribution probably reflects ^{3}H-ethanol distribution, as discussed for chicken legs.

In the case of ^{3}H-PG, ^{14}C-CA and ^{14}C-EDTA, the distribution into fractions are as expected and are similar to those reported for freeze-dried beef stew.

For the storage stability studies, application of PG and CA by spraying before freezing was chosen. This method was selected because it was relatively easy to control the applied amount. PG was chosen as the phenolic antioxidant to complete the experimental design of using each phenolic antioxidant in at least one product and because it is more polar resulting in perhaps greater penetration into the high A_w carrot pieces. Also PG showed the highest retention of all the phenolic antioxidants when added by spraying before freezing (Table 8). CA was selected as the water soluble, chelating-type antioxidant because it was completely retained through processing. Sensory evaluation data are summarized in Figs. 37, 38 and 39 and the chemical stability data are summarized in Figs. 40, 41, 42 and 43.

The overall acceptability scores (Fig. 37) decline rapidly during the first three months of storage and then stabilize. Incorporation of 150 ppm PG and CA had no effect on the stability of the freeze-dried carrots. If it is assumed that an overall acceptability score of 4.0 represents the cutoff point for acceptable product, then the storage life for freeze-dried carrots as prepared and stored in this study is about one month.

Fig. 38 shows that there was a significant and progressive increase in relative oxidized flavor intensity with storage time. The flavor was described as violet-like suggesting that β-ionone, a degradation product from the oxidation of β-carotene, may be involved. The intensity of other off-flavors also increased with storage time (Fig. 39); however, it is suggested that this may be the result of confusion by the panel of the typical oxidized flavor and the β-ionone flavor. In any event, the antioxidants did not influence the development of these flavors in freeze-dried carrots.

The TBA values (Fig. 40) showed a slight increase throughout the storage period indicating the formation of malonaldehyde or other products which are measured by the TBA test. The peroxide values (Fig. 41) could not be measured during the first two months due to interference by β-carotene. After two months, the β-carotene was sufficiently reduced by oxidation to enable determination of the peroxide number. Peroxide numbers between the sample with antioxidant and the sample without do not appear to be different but both are higher than those reported for the other products except beef stew. These higher values suggest significant peroxidation of the β-carotene.

For chemical analysis, PG was attempted but the antioxidant could not be measured because of color interference and difficulty in TLC preparation.

In conclusion, the antioxidants and method of application used on freeze-dried carrots in this study did not result in increased storage life when stored at 32 C in air. Freeze-dried carrots under these conditions exhibited a storage life of less than one month.

G. Other Observations.

In the course of this study, it became readily apparent that for those products which were fried in oil during preparation (breaded chicken legs and breaded fish sticks) it was necessary to ascertain if oxidized flavor might be transmitted to the product directly from the oil. Therefore, cottonseed frying oil was prepared both with and without the appropriate antioxidants (combination of BHT and EDTA for chicken legs, and BHA and CA for fish sticks) and exposed to frying conditions (204 C) for up to 135 min. Samples of cottonseed oil were removed periodically and analyzed for peroxide value. The results, shown in Tables 37 and 38 for chicken legs and fish sticks, respectively, show low peroxide values for frying oil both with and without antioxidants indicating little development of peroxides and suggesting low levels of oxidation. Further the peroxide values remained fairly constant throughout the frying period. Thus oxidized flavors of chicken legs and fish sticks which were observed by the technological panel were not transferred directly from the frying oil but developed once the product was placed in storage.

A further consideration in protecting frying oils by addition of antioxidants is the retention of the antioxidant in the oil. This is important not only for determining the rate of addition of antioxidant as makeup for that volatilized during frying but also, in the case of addition of antioxidant via the drying oil, to insure adequate levels of antioxidant delivery to the food system to provide the desired amount (200 ppm, fat basis). Based on volatility considerations, the phenolic antioxidants (BHT, BHA and PG) would appear to be particularly vulnerable to losses from frying oil during heating. Therefore, an experiment was performed using ^{14}C-BHT in cottonseed oil heated to 204 C for up to six hours. ^{3}H-BHA and ^{3}H-PG were not used because of anticipated tritium exchange at the high temperatures. Data on the retention of ^{14}C-BHT, shown in Table 39, indicate that the loss of ^{14}C-BHT is rapid and extensive, about 70% being lost within 30 min. With BHA, it would be anticipated that losses would be even greater because of the higher volatility (lower melting point) and increased polarity. Thus in designing methods of adding these antioxidants via frying oil, consideration of volatilization losses would have to be made and accounted for.

V. Summary

In determining the efficacy of antioxidants in preventing development of oxidative deterioration in products, it is necessary to determine the location, site or association of the antioxidant. One phase of this study was designed to utilize radioactive antioxidants to determine efficiency of various antioxidant delivery systems and distribution and penetration of antioxidant in the food systems. As pointed out in the specific discussion sections on each product, this technique was quite acceptable for determining retention of antioxidants through subsequent processing provided changes in the antioxidant were considered. Two important considerations are: 1) tritium exchange may occur between the tritium labeled antioxidants (^{3}H-BHA and ^{3}H-PG) and the food milieu, and 2) the measured radioactivity may be associated with antioxidant which is no longer capable of performing its function. For the distribution into various fractions, using radioactive antioxidants appears on the surface to provide an excellent means for determining the associative relationship between antioxidant and free lipid, bound lipid, aqueous and solid fractions. However, some caution must be exercised when interpreting these data. These determinations may reflect partitioning effects of the antioxidant between the extracting solvent and the food milieu and not reflect actual associations. This point is extremely important and has been repeatedly stated in the Discussion section. In analyzing the results from the distribution of radioactive antioxidant into various fractions, therefore, it is necessary to look for the exceptional data for interpretation rather than the norm. The norm may reflect partitioning effects, whereas exceptional data reflect unusual associations. In addition, comparisons between products should be done with extreme caution.

For the storage stability studies, the only product in which the antioxidant combination appeared to delay oxidative deterioration was frankfurters. In the case of chicken legs and fish sticks, the products were acceptable throughout the 6 mo storage period indicating that oxidative deterioration was not yet a factor in determining acceptability. Since the products without antioxidants were also acceptable the effectiveness of the combination of antioxidant could not be assessed. By extending the storage period to up to one year, perhaps protection would be evident. For frankfurters without antioxidant the storage life at -26 C is about 1 mo; whereas, with antioxidants, the storage life was extended to at least 6 mo. Freeze-dried pork chops were unacceptable almost immediately after manufacture and the antioxidants did not provide any noticeable beneficial effect. Rapid development of oxidative deterioration in freeze-dried products has long been recognized as a major problem (2), and with the large surface area for oxygen exposure, levels of antioxidants that are found effective for frozen, high A_w foods (150-200 ppm) may not be adequate for freeze-dried products. For freeze-dried carrots and beef stew,

the storage life was less than one month and presence of antioxidants did not affect onset of oxidative deterioration.

Attempts to chemically measure levels of BHT, BHA, PG and EDTA were largely unsuccessful. Although this study was not designed to test methods of antioxidant analysis, methods were chosen that are generally used with food products. The inability to accurately measure the antioxidants suggests that there is a need to develop procedures and methodology which can be used for antioxidants in food products.

Finally this study suggests some very definite directions for future studies in antioxidant application technology and the role of antioxidants in preventing oxidative deterioration. A detailed investigation involving selected antioxidants and one product which is susceptible to lipid oxidation (e.g. fish or fish sticks) could be designed to provide information on the role and active site location for antioxidant effectiveness. Once this has been delineated it would be appropriate to develop studies which would consider antioxidant delivery systems that would achieve the desired distribution and penetration. Innovative methods such as those discussed and tried in this study could then be selected on a rational basis rather than on a try-it-and-see basis. Application of phenolic antioxidants to food systems via frying oil, immersion freezing in Freon^{R} Food Freezant and freeze-dry vacuum release are all methods for which developmental technology is needed, but a determination as to whether these methods are effective in distributing the antioxidant to the active sites should be carried out before technology development is initiated.

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VII. Appendix A

TABLE A1 TECHNOLOGICAL PANEL SENSORY EVALUATION OF CHICKEN LEGS DURING STORAGE AT -26 C FOR 6 MO.

	Time (mo)						
	0	1	2	3	4	5	6
	(———			Mean Score			———)
Overall Acceptability[1]							
WO/Anti[4]	5.69^{a}	5.65^{a}	4.74^{a}	5.18^{a}	4.65^{a}	5.07^{a}	5.63^{a}
W/Anti	5.19^{a}	5.23^{a}	4.74^{a}	5.24^{a}	5.02^{a}	4.78^{a}	5.15^{b}
Ref/Com	--	--	4.85^{a}	5.82^{b}	5.63^{b}	4.96^{a}	6.20^{c}
F-Value	ns[5]	ns	ns	s[6]	s	ns	s
LSD 5%	.52	.47	.57	.45	.56	.71	.47
N[7] =	27	24	23	17	23	23	20
Intensity of Oxidized Flavor[2]							
WO/Anti	1.63^{a}	1.44^{a}	2.04^{a}	$1.59^{a,b}$	1.87^{a}	2.27^{a}	1.53^{a}
W/Anti	1.70^{a}	1.54^{a}	1.88^{a}	1.65^{a}	1.72^{a}	2.27^{a}	1.78^{a}
Ref/Com	--	--	1.65^{a}	1.27^{b}	1.09^{b}	1.56^{b}	1.08^{b}
F-Value	ns	ns	ns	s	s	s	s
LSD 5%	.29	.48	.57	.32	.43	.58	.38
N =	27	24	24	17	23	24	20
Intensity of Other Off-Flavors[3]							
WO/Anti	1.46^{a}	1.27^{a}	1.19^{a}	1.40^{a}	1.39^{a}	1.90^{a}	$1.40^{a,b}$
W/Anti	1.69^{a}	1.27^{a}	1.29^{a}	1.33^{a}	1.30^{a}	1.63^{a}	1.73^{a}
Ref/Com	--	--	1.27^{a}	1.37^{a}	1.11^{a}	1.50^{a}	1.08^{b}
F-Value	ns	ns	ns	ns	ns	ns	s
LSD 5%	.46	.28	.18	.27	.28	.49	.35
N =	27	24	24	17	22	24	20

[1] Overall Acceptability Scale: 1 = Extremely Unacceptable, 7 = Extremely Acceptable
[2] Intensity of Oxidation Scale: 1 = None, 7 = Extreme
[3] Intensity of Off-Flavor Scale: 1 = None, 7 = Extreme
[4] BHT and EDTA
[5] ns = not significant
[6] s = significant
[7] N = number of judges
a,b,c Mean scores with similar superscripts within the same comparison are not significantly different at the 5% level.

TABLE A2 TECHNOLOGICAL PANEL SENSORY EVALUATION OF FISH STICKS DURING STORAGE AT -26 C FOR 6 MO.

	Time (mo)						
	0	1	2	3	4	5	6
	(Mean Score)						
Overall Acceptability[1]							
WO/Anti[4]	5.23^{a}	5.38^{a}	4.94^{a}	4.69^{a}	4.58^{a}	4.14^{a}	4.68^{a}
W/Anti	5.38^{a}	4.98^{a}	5.07^{a}	4.00^{b}	4.35^{a}	$4.75^{a,b}$	4.54^{a}
Ref/Com	--	--	--	5.44^{c}	5.45^{b}	5.33^{b}	4.54^{a}
F-Value	ns[5]	ns	ns	s[6]	s	s	ns
LSD 5%	.47	.52	.54	.60	.62	.90	.92
N[7] =	24	25	23	18	20	18	14
Intensity of Oxidized Flavor[2]							
WO/Anti	1.54^{a}	1.30^{a}	1.50^{a}	2.45^{a}	2.12^{a}	2.58^{a}	1.85^{a}
W/Anti	1.65^{a}	1.20^{a}	1.50^{a}	2.58^{a}	2.29^{a}	2.03^{b}	1.92^{a}
Ref/Com	--	--	--	1.58^{b}	1.41^{b}	1.47^{c}	1.58^{a}
F-Value	ns	ns	ns	s	s	s	ns
LSD 5%	.18	.27	.37	.60	.58	.51	.52
N =	24	25	23	19	21	19	13
Intensity of Other Off-Flavors[3]							
WO/Anti	1.73^{a}	1.24^{a}	1.24^{a}	1.90^{a}	$1.58^{a,b}$	1.64^{a}	1.25^{a}
W/Anti	1.58^{a}	1.74^{a}	1.41^{a}	2.18^{a}	1.78^{a}	2.04^{a}	1.79^{b}
Ref/Com	--	--	--	1.34^{b}	1.22^{b}	1.43^{a}	1.25^{c}
F-Value	ns	ns	ns	s	s	ns	ns
LSD 5%	.31	.58	.30	.52	.39	.81	.52
N =	24	25	23	19	18	14	12

[1] Overall Acceptability Scale: 1 = Extremely Unacceptable, 7 = Extremely Acceptable
[2] Intensity of Oxidation Scale: 1 = None, 7 = Extreme
[3] Intensity of Off-Flavor Scale: 1 = None, 7 = Extreme
[4] BHA and CA
[5] ns = not significant
[6] s = significant
[7] N = number of judges
a,b,c Mean scores with similar superscripts within the same comparison are not significantly different at the 5% level.

TABLE [illegible].3 TECHNOLOGICAL PANEL SENSORY EVALUATION OF FRANKFURTERS DURING STORAGE AT -26 C FOR 6 MO.

	Time (mo)						
	0	1	2	3	4	5	6
	(Mean Score)						
Overall Acceptability[1]							
WO/Anti[4]	4.52^{a}	4.43^{a}	3.05^{a}	2.87^{a}	3.32^{a}	2.96^{a}	3.14^{a}
W/Anti	5.20^{b}	5.09^{b}	3.90^{a}	4.16^{b}	3.86^{a}	4.14^{b}	3.78^{a}
Ref/Com	--	--	5.80^{b}	5.90^{c}	5.82^{b}	6.11^{c}	5.92^{b}
F-Value	s[6]	s	s	s	s	s	s
LSD 5%	.38	.53	1.02	.65	.75	.77	.74
N[7] =	23	28	10	19	14	14	18
Intensity of Oxidized Flavor[2]							
WO/Anti	1.61^{a}	2.36^{a}	4.20^{a}	3.79^{c}	3.31^{a}	3.75^{a}	3.73^{a}
W/Anti	1.30^{b}	1.61^{b}	2.55^{b}	2.63^{b}	2.78^{a}	2.94^{b}	3.43^{a}
Ref/Com	--	--	1.05^{c}	1.03^{c}	1.06^{b}	1.06^{c}	1.15^{b}
F-Value	s	s	s	s	s	s	s
LSD 5%	.29	.62	1.05	.74	.80	.79	.54
N =	23	28	10	19	14	14	18
Intensity of Other Off-Flavors[3]							
WO/Anti	2.11^{a}	1.86^{a}	2.72^{a}	2.35^{a}	2.00^{a}	2.66^{a}	2.42^{a}
W/Anti	1.50^{b}	1.57^{a}	1.67^{b}	1.62^{b}	1.40^{b}	2.09^{a}	1.69^{b}
Ref/Com	--	--	1.00_{b}	1.09^{b}	1.13^{b}	1.03^{b}	1.17^{b}
F-Value	s	ns[5]	s	s	s	s	s
LSD 5%	.40	.35	.59	.59	.59	.72	.54
N =	23	28	10	19	14	14	18

[1] Overall Acceptability Scale: 1 = Extremely Unacceptable, 7 = Extremely Acceptable
[2] Intensity of Oxidation Scale: 1 = None, 7 = Extreme
[3] Intensity of Off-Flavor Scale: 1 = None, 7 = Extreme
[4] BHT and EDTA
[5] ns = not significant
[6] s = significant
[7] N = number of judges
a,b,c Mean scores with similar superscripts within the same comparison are not significantly different at the 5% level.

TABLE A4 TECHNOLOGICAL PANEL SENSORY EVALUATION OF FREEZE-DRIED PORK CHOPS DURING STORAGE IN AIR AT 32 C FOR 4 MO.

	Time (mo)				
	0	1	2	3	4
	(Mean Score)				
Overall Acceptability[1]					
WO/Anti[4]	3.75[a]	2.78[a]	2.29[a]	2.47[a]	1.96[a]
W/Anti	3.72[a]	2.67[a]	2.37[a]	2.34[a]	2.14[a]
Ref/Com	--	5.83[b]	5.47[b]	5.63[b]	5.79[b]
F-Value	ns[5]	s[6]	s	s	s
LSD 5%	.53	.58	.61	.66	.66
N[7] =	22	9	19	16	14
Intensity of Oxidized Flavor[2]					
WO/Anti	1.84[a]	2.89[a]	3.34[a]	3.94[a]	3.84[a]
W/Anti	1.68[a]	3.56[a]	2.95[a]	3.29[a]	3.62[a]
Ref/Com	--	1.00[b]	1.29[b]	1.25[b]	1.25[b]
F-Value	ns	s	s	s	s
LSD 5%	.80	1.10	.60	.69	.70
N =	22	9	19	16	14
Intensity of Other Off-Flavors[3]					
WO/Anti	1.59[a]	2.06[a]	2.27[a]	2.27[a]	2.25[a]
W/Anti	1.52[a]	2.11[a]	2.09[a]	2.20[a]	2.38[a]
Ref/Com	--	1.06[b]	1.21[b]	1.13[b]	1.19[b]
F-Value	ns	s	s	s	s
LSD 5%	.66	.81	.47	.63	.61
N =	22	9	19	16	14

[1] Overall Acceptability Scale: 1 = Extremely Unacceptable, 7 = Extremely Acceptable

[2] Intensity of Oxidation Scale: 1 = None, 7 = Extreme

[3] Intensity of Off-Flavor Scale: 1 = None, 7 = Extreme

[4] BHA and CA

[5] ns = not significant

[6] s = significant

[7] N = number of judges

a,b Mean scores with similar superscripts within the same comparison are not significantly different at the 5% level.

TABLE A5 TECHNOLOGICAL PANEL SENSORY EVALUATION OF FREEZE-DRIED BEEF STEW DURING STORAGE IN AIR AT 32 C FOR 4 MO.

	Time (mo)				
	0	1	2	3	4
	(Mean Score)				
Overall Acceptability[1]					
WO/Anti[4]	4.96^a	4.19^a	2.00^a	3.18^a	1.73^a
W/Anti	5.00^a	3.96^a	3.06^b	2.65^a	2.05^a
Ref/Com	--	--	5.65^c	5.50^b	6.11^b
F-Value	ns[5]	ns	s[6]	s	s
LSD 5%	.36	.58	.48	.64	.40
N[7] =	26	27	17	17	22
Intensity of Oxidized Flavor[2]					
WO/Anti	1.50^a	2.37^a	4.94^a	4.07^a	5.08^a
W/Anti	1.52^a	2.48^a	3.53^b	4.65^a	5.25^a
Ref/Com	--	--	1.25^c	1.44^b	1.42^b
F-Value	ns	ns	s	s	s
LSD 5%	.07	.61	.68	.62	.78
N =	26	27	18	23	24
Intensity of Other Off-Flavors[3]					
WO/Anti	1.56^a	1.59^a	2.75^a	2.28^a	3.55^a
W/Anti	1.42^a	2.02^a	2.04^a	2.53^a	3.21^a
Ref/Com	--	--	1.14^b	1.48^b	1.18^b
F-Value	ns	ns	s	s	s
LSD 5%	.15	.54	.87	.73	.81
N =	26	27	14	20	22

[1] Overall Acceptability Scale: 1 = Extremely Unacceptable, 7 = Extremely Acceptable

[2] Intensity of Oxidation Scale: 1 = None, 7 = Extreme

[3] Intensity of Off-Flavor Scale: 1 = None, 7 = Extreme

[4] BHA and CA

[5] ns = not significant

[6] s = significant

[7] N = number of judges

a,b Mean scores with similar superscripts within the same comparison are not significantly different at the 5% level.

TABLE A6 TECHNOLOGICAL PANEL SENSORY EVALUATION OF FREEZE DRIED CARROTS DURING STORAGE IN AIR AT 32 C FOR 6 MO.

	Time (mo)						
	0	1	2	3	4	5	6
	(————			Mean Score			————)
Overall Acceptability[1]							
WO/Anti[4]	4.56^{a}	4.00^{a}	3.26^{a}	3.00^{a}	2.27^{a}	2.42^{a}	2.75^{a}
W/Anti	4.56^{a}	4.12^{a}	3.45^{a}	2.90^{a}	2.62^{a}	2.50^{a}	2.75^{a}
Ref/Com	--	--	--	5.55^{b}	5.38^{b}	5.19^{b}	5.39^{b}
F-Value	ns[5]	ns	ns	s[6]	s	s	s
LSD 5%	.43	.48	.52	.60	.72	.63	.79
N[7] =	25	25	21	19	17	18	14
Intensity of Oxidized Flavor[2]							
WO/Anti	1.58^{a}	1.72^{a}	2.31^{a}	3.33^{a}	3.58^{a}	3.79^{a}	3.86^{a}
W/Anti	1.58^{a}	1.98^{a}	2.07^{a}	3.19^{a}	3.28^{a}	3.63^{a}	3.86^{a}
Ref/Com	--	--	--	1.22^{b}	1.10^{b}	1.24^{b}	1.21^{b}
F-Value	ns	ns	ns	s	s	s	s
LSD 5%	.26	.37	.77	.81	.78	.70	.87
N =	25	25	21	20	20	19	14
Intensity of Other Off-Flavors[3]							
WO/Anti	1.54^{a}	1.42^{a}	1.81^{a}	1.97^{a}	2.24^{a}	1.92^{a}	2.58^{a}
W/Anti	1.36^{a}	1.62^{a}	1.62^{a}	2.13^{a}	2.32^{a}	1.97^{a}	$1.96^{a,b}$
Ref/Com	--	--	--	1.20^{b}	1.21^{b}	1.31^{b}	1.08^{b}
F-Value	ns	ns	ns	s	s	s	s
LSD 5%	.14	.51	.71	.63	.71	.53	.95
N =	25	25	21	15	19	18	13

[1] Overall Acceptability Scale: 1 = Extremely Unacceptable, 7 = Extremely Acceptable
[2] Intensity of Oxidation Scale: 1 = None, 7 = Extreme
[3] Intensity of Off-Flavor Scale: 1 = None, 7 = Extreme
[4] PG and CA
[5] ns = not significant
[6] s = significant
[7] N = number of judges
a,b Mean scores with similar superscripts within the same comparison are not significantly different at the 5% level.

TABLE A7 THIOBARBITURIC ACID (TBA) NUMBERS, PEROXIDE VALUES, AND ULTRAVIOLET SPECTRAL ABSORBANCES FOR CHICKEN LEGS CONTAINING BHT AND EDTA DURING STORAGE AT -26 C FOR 6 MO.

Storage Time (mo)	TBA Number (mg malonaldehyde / kg wet wt)		Peroxide Value (meq/kg fat)		Absorbance ($A_{1\ cm}^{1\ \%\ fat}$) 232 nm		Absorbance ($A_{1\ cm}^{1\ \%\ fat}$) 268 nm	
	WO/Anti	W/Anti	WO/Anti	W/Anti	WO/Anti	W/Anti	WO/Anti	W/Anti
Initial	1.0	0.6	0.9	0.7	5.7	7.7	2.4	1.8
1	0.8	0.9	ND	ND	6.5	7.6	2.3	1.6
2	0.6	0.6	ND	ND	6.7	8.9	2.2	2.1
3	0.7	0.5	2.6	4.2	6.1	7.4	2.2	1.4
4	0.6	0.7	2.5	2.0	7.6	8.7	2.3	1.8
5	0.8	0.8	2.8	2.1	7.6	8.2	2.4	1.6
6	0.8	0.8	3.3	1.9	7.2	8.8	2.3	1.8

[1] ND = not detected.

TABLE A8 THIOBARBITURIC ACID (TBA) NUMBERS, PEROXIDE VALUES, AND ULTRAVIOLET SPECTRAL ABSORBANCES FOR FISH STICKS CONTAINING BHA AND CA DURING STORAGE AT -26 C FOR 6 MO.

Storage Time (mo)	TBA Number ($\frac{\text{mg malonaldehyde}}{\text{kg wet wt}}$)		Peroxide Value (meq/kg fat)		Absorbance ($A_{1\ cm}^{1\ \%\ fat}$) 232 nm		268 nm	
	WO/Anti	W/Anti	WO/Anti	W/Anti	WO/Anti	W/Anti	WO/Anti	W/Anti
Initial	0.2	0.6	3.6	3.1	10.9	9.5	4.2	3.9
1	0.2	0.2	ND	ND	11.2	9.0	4.1	3.5
2	0.2	0.2	ND	ND	11.9	9.7	3.9	3.4
3	0.2	0.2	4.7	5.3	12.1	9.5	4.9	3.7
4	0.2	0.2	1.2	1.3	13.3	10.4	4.4	3.7
5	0.2	0.2	1.7	1.1	12.6	10.8	4.5	4.5
6	0.2	0.2	0.7	0.6	13.6	12.0	4.1	3.8

[1]ND = not detected.

TABLE A9 THIOBARBITURIC ACID (TBA) NUMBERS, PEROXIDE VALUES, AND ULTRAVIOLET SPECTRAL ABSORBANCES FOR FRANKFURTERS CONTAINING BHA AND EDTA DURING STORAGE AT -26 C FOR 6 MO.

Storage Time (mo)	TBA Number (mg malonaldehyde / kg wet wt)		Peroxide Value (meq/kg fat)		Absorbance ($A_{1\ cm}^{1\ \%\ fat}$) 232 nm		Absorbance 268 nm	
	WO/Anti	W/Anti	WO/Anti	W/Anti	WO/Anti	W/Anti	WO/Anti	W/Anti
Initial	0.1	0.4	ND	ND	3.7	3.9	0.3	0.3
1	0.6	0.4	2.1	1.2	3.5	3.7	0.3	0.3
2	0.7	0.4	1.3	1.1	3.5	4.1	0.3	0.3
3	0.6	0.3	1.5	1.3	4.2	4.0	0.3	0.3
4	0.7	0.5	1.0	0.8	3.4	3.6	0.3	0.3
5	0.8	0.6	1.0	1.1	3.3	3.4	0.3	0.3
6	0.8	0.8	ND	ND	3.7	3.4	0.3	0.3

[1]ND = not detected.

TABLE A10 THIOBARBITURIC ACID (TBA) NUMBERS, PEROXIDE VALUES, AND ULTRAVIOLET SPECTRAL ABSORBANCES FOR FREEZE-DRIED PORK CHOPS CONTAINING BHA AND CA DURING STORAGE AT 32 C FOR 6 MO.

Storage Time (mo)	TBA Number (mg malonaldehyde / kg wet wt)		Peroxide Value (meq/kg fat)		Absorbance ($A_{1\ cm}^{1\ \%\ fat}$) 232 nm		268 nm	
	WO/Anti	W/Anti	WO/Anti	W/Anti	WO/Anti	W/Anti	WO/Anti	W/Anti
Initial	1.2	1.2	14.7	12.2	4.3	3.1	0.8	0.7
1	1.8	1.7	11.2	9.9	5.5	4.7	1.2	1.1
2	1.1	1.4	2.3	2.0	4.1	4.8	0.5	0.7
3	1.5	1.6	3.5	3.3	6.3	4.1	0.9	1.5
4	1.5	1.9	2.6	2.7	5.4	5.8	1.3	1.5
5	1.4	2.1	ND	ND	5.5	5.2	1.1	1.2
6	1.7	1.8	ND	ND	4.9	4.4	1.0	0.9

[1] ND = not detected.

TABLE A11 THIOBARBITURIC ACID (TBA) NUMBERS, PEROXIDE VALUES, AND ULTRAVIOLET SPECTRAL ABSORBANCES FOR FREEZE-DRIED BEEF STEW CONTAINING BHA AND CA DURING STORAGE AT 32 C FOR 6 MO.

Storage Time (mo)	TBA Number (mg malonaldehyde / kg wet wt)		Peroxide Value (meq/kg fat)		Absorbance ($A_{1\ cm}^{1\ \%\ fat}$) 232 nm		Absorbance ($A_{1\ cm}^{1\ \%\ fat}$) 2[illegible]8 nm	
	WO/Anti	W/Anti	WO/Anti	W/Anti	WO/Anti	W/Anti	WO/Anti	W/Anti
Initial	4.3	3.5	40.0	22.4	14.2	11.8	2.6	2.7
1	3.7	2.5	151.6	117.4	26.0	17.4	3.6	3.3
2	3.2	2.9	129.6	63.1	24.0	16.7	4.7	3.7
3	2.7	2.1	79.1	34.9	20.7	15.8	5.6	4.5
4	2.8	2.[illegible]	94.0	123.0	19.3	18.5	5.7	5.1
5	3.3	2.2	157.8	76.6	21.4	19.5	6.2	6.4
6	2.6	2.3	48.7	42.0	26.5	15.3	5.4	2.9

[1] ND = not detected.

TABLE A12 THIOBARBITURIC ACID (TBA) NUMBERS, PEROXIDE VALUES, AND ULTRAVIOLET SPECTRAL ABSORBANCES FOR FREEZE-DRIED CARROTS CONTAINING PG AND CA DURING STORAGE AT 32 C FOR 6 MO.

Storage Time (mo)	TBA Number (mg malonaldehyde / kg wet wt)		Peroxide Value (meq/kg fat)		Absorbance ($A_{1\,cm}^{1\,\%\,fat}$) 232 nm		268 nm	
	WO/Anti	W/Anti	WO/Anti	W/Anti	WO/Anti	W/Anti	WO/Anti	W/Anti
Initial	0.6	0.7	ND	ND	27.2	28.0	29.7	31.6
1	0.8	1.0	ND	ND	21.9	25.0	18.4	22.0
2	1.4	1.2	100.2	101.7	35.6	34.4	22.7	20.4
3	1.1	1.1	138.0	147.2	39.0	39.1	18.6	18.4
4	2.0	1.9	165.1	209.8	45.0	40.5	23.1	21.1
5	1.6	1.5	182.4	281.1	44.8	39.2	22.8	20.8
6	1.8	2.1	150.0	202.8	47.3	43.1	24.5	20.5

[1] ND = not detected.

TABLE A13 INTERCEPT AND COEFFICIENTS FROM REGRESSION ANALYSIS FOR CHICKEN LEGS DURING STORAGE AT -26 C FOR 6 MO.

	Coefficients and Intercepts		
	α	B_1X	B_2X^2
Overall Acceptability			
WO/Anti	5.842	-.6018	.0918
W/Anti	5.216	-.1307	.0174
Ref/Com	4.756	.184	
Oxidized Flavor			
WO/Anti	1.640	.025	
W/Anti	1.614	.0578	
Ref/Com	1.664	-.082	
Off-Flavor			
WO/Anti	1.293	.0457	
W/Anti	1.372	.0304	
Ref/Com	1.366	-.025	
TBA			
WO/Anti	.9754	-.1950	.0280
W/Anti	.5621	.0421	
Peroxide			
WO/Anti	.0921	.5436	
W/Anti	.5143	.3429	
SA 232			
WO/Anti	5.926	.2821	
W/Anti	7.750	.1500	
SA 268			
WO/Anti	2.370	-.0917	.0153
W/Anti	1.823	-.0946	.0129

TABLE A14 INTERCEPT AND COEFFICIENTS FROM REGRESSION ANALYSIS FOR FISH STICKS DURING STORAGE AT -26 C FOR 6 MO.

	Coefficients and Intercepts		
	α	B_1X	B_2X^2
Overall Acceptability			
WO/Anti	5.2868	-.1604	
W/Anti	5.1207	-.1321	
Ref/Com	6.459	-.282	
Oxidized Flavor			
WO/Anti	1.4654	.1468	
W/Anti	1.532	.1164	
Ref/Com	2.813	-.624	.07
Off-Flavor			
WO/Anti	1.475	.0721	-.0138
W/Anti	1.467	.295	-.0493
Ref/Com	1.052	.129	-.015
TBA			
WO/Anti	.231	-.0371	.005
W/Anti	.468	-.182	.0233
Peroxide			
WO/Anti	2.1275	-.1496	
W/Anti	2.0354	-.1432	
SA 232			
WO/Anti	10.91	.439	
W/Anti	9.295	-.200	.104
SA 268			
WO/Anti	4.13	.0682	
W/Anti	3.62	.058	

TABLE A15 INTERCEPT AND COEFFICIENTS FROM REGRESSION ANALYSIS FOR FRANKFURTERS DURING STORAGE AT -26 C FOR 6 MO.

	Coefficients and Intercepts		
	α	B_1X	B_2X^2
Overall Acceptability			
WO/Anti	4.659	-.7939	.0918
W/Anti	4.97	-.2214	
Ref/Com	5.73	.045	
Oxidized Flavor			
WO/Anti	1.7119	1.080	-.1308
W/Anti	1.476	.3314	
Ref/Com	1.008	.018	
Off-Flavor			
WO/Anti	2.109	.0646	
W/Anti	1.505	.0479	
Ref/Com	.972	.028	
TBA			
WO/Anti	.2960	.1003	
W/Anti	.4095	-.0878	.0254
Peroxide			
WO/Anti	1.24	-.088	
W/Anti	.87	-.023	
SA 232			
WO/Anti	3.695	-.0203	
W/Anti	3.805	.1314	-.0371
SA 268			
WO/Anti	.3219	-.0182	.0027
W/Anti	.2766	.0032	-.0005

TABLE A16 INTERCEPT AND COEFFICIENT FROM REGRESSION ANALYSIS FOR FREEZE DRIED PORK CHOPS DURING STORAGE IN AIR AT 32 C FOR 6 MO.

	Coefficients and Intercepts		
	α	B_1X	B_2X^2
Overall Acceptability			
WO/Anti	3.428	-.389	
W/Anti	3.627	-.912	.141
Ref/Com	6.32	-.646	.13
Oxidized Flavor			
WO/Anti	1.853	1.119	-.154
W/Anti	2.298	.421	
Ref/Com	.6575	.4335	-.0725
Off-Flavor			
WO/Anti	1.612	.493	-.085
W/Anti	1.698	.181	
Ref/Com	1.07	.031	
TBA			
WO/Anti	1.32	.042	
W/Anti	1.342	.1107	
Peroxide			
WO/Anti	14.66	-5.564	.533
W/Anti	12.27	-4.396	.4012
SA 232			
WO/Anti	4.382	.6300	-.0864
W/Anti	3.370	1.004	-.1314
SA 268			
WO/Anti	.8246	.0475	
W/Anti	.6114	.4146	-.0596

TABLE A17 INTERCEPT AND COEFFICIENTS FROM RECRESSION ANALYSIS FOR FREEZE DRIED BEEF STEW DURING STORAGE IN AIR AT 32 C FOR 6 MO.

	Coefficients and Intercepts		
	α	B_1X	B_2X^2
Overall Acceptability			
WO/Anti	4.706	-.747	
W/Anti	4.982	-1.11	.0979
Ref/Com	5.063	.23	
Oxidized Flavor			
WO/Anti	1.82	.886	
W/Anti	1.56	.163	
Ref/Com	1.115	.085	
Off-Flavor			
WO/Anti	1.412	.467	
W/Anti	1.426	.409	
Ref/Com	1.207	.02	
TBA			
WO/Anti	3.903	-.225	
W/Anti	3.070	-.1514	
Peroxide			
WO/Anti	-	-	
W/Anti	-	-	
SA 232			
WO/Anti	19.26	.8214	
W/Anti	12.6	2.96	-.3964
SA 268			
WO/Anti	2.472	1.483	-.161
W/Anti	2.19	1.48	-.1970

TABLE A18 INTERCEPT AND COEFFICIENT FROM REGRESSION ANALYSIS FOR FREEZE DRIED CARROTS DURING STORAGE IN AIR AT 32 C FOR 6 MO.

	Coefficients and Intercepts			
	α	B_1X	B_2X^2	B_3X^3
Overall Acceptability				
WO/Anti	4.6802	-.9107	.09476	
W/Anti	4.6905	-.8207	.0802	
Ref/Com	5.674	-.067		
Oxidized Flavor				
WO/Anti	1.569	.4375		
W/Anti	1.583	.4054		
Ref/Com	1.143	.0110		
Off-Flavor				
WO/Anti	1.438	.1625		
W/Anti	1.511	.1143		
Ref/Com	.177	.514	-.06	
TBA				
WO/Anti	.7017	.2089		
W/Anti	.7628	.1971		
Peroxide				
WO/Anti	-8.5262	20.0694	17.2071	-2.7028
W/Anti	-1.5952	-21.509	40.5369	-5.1778
SA 232				
WO/Anti	27.06	2.850		
W/Anti	24.88	4.125		
SA 268				
WO/Anti	27.01	-4.400	.6952	
W/Anti	29.49	-5.557	.7214	